Universidade Estadual de Londrina

EDUARDO ANTÔNIO DE ALMEIDA ROSSIGNOLO

AVALIAÇÃO DA hCG COMO SUPORTE GONADOTRÓFICO EM PROTOCOLOS DE TRANSFERÊNCIA DE EMBRIÃO EM TEMPO FIXO EM GADO DE CORTE

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Orientador: Prof. Dr. Fábio Morotti

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Londrina, 25 de setembro de 2023.

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A minha noiva, pelo suporte emocional, incentivo e parceria em todas as adversidades que encontrei no concílio da carreira profissional com as atividades acadêmicas.

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RESUMO

Este estudo comparou o desempenho reprodutivo de receptoras de embriões tratadas com um protocolo de transferência de embriões em tempo fixo (TETF) usando gonadotrofina coriônica humana (hCG) ou gonadotrofina coriônica equina (eCG). Em um dia aleatório do ciclo estral (Dia -10) receptoras da raça *Bos taurus indicus* (n = 341; 194 vacas nulíparas e 147 multíparas) com escore de condição corporal entre 3,0 e 4,0, foram submetidas ao protocolo TETF que consiste em uma injeção intramuscular i.m. de 2,0 mg de benzoato de estradiol (BE) e inserção de dispositivo intravaginal de progesterona (P4) que permaneceu até o dia -2,5. No mesmo dia (-2,5), os animais receberam uma injeção i.m. de 150 mg de D-cloprostenol e 1 mg de cipionato de estradiol e foram divididos aleatoriamente em dois grupos: o grupo eCG (n = 179), no qual as fêmeas receberam injeção i.m. 300 UI de eCG e o grupo hCG (n = 162), no qual as fêmeas receberam 150 UI de hCG. Em seguida, no D0 os animais foram avaliados quanto a intensidade do estro, diâmetro folicular (DF) por ultrassonografia modo B e no D7 foi avaliado a qualidade do corpo lúteo (CL) por ultrassonografia (modo B e doppler colorido) para selecionar as receptoras elegíveis a receber a transferência de um embrião produzidos *in vitro*. O diagnóstico de gestação foi avaliado 23 dias após a transferência. Os dados contínuos foram analisados por ANOVA usando um modelo de efeitos mistos e o teste de Tukey. As taxas foram analisadas por meio de um modelo de regressão logística. O DF no D0 do protocolo TETF foi influenciado pela interação entre o tratamento gonadotrófico e categoria (P = 0,01), sendo que as receptoras nulíparas tratadas com hCG apresentaram o menor diâmetro. O tratamento com hCG e eCG resultou em alta taxa de expressão de estro, no entanto, a proporção de fêmeas com estro de alta intensidade foi maior no grupo hCG (79,84 vs. 68,61%, respectivamente; P = 0,03). A taxa de utilização (receptoras com CL) apresentou tendência (P = 0,06) a ser influenciada pela interação entre o tratamento gonadotrófico e categoria, sendo que as receptoras nulíparas tratadas com hCG apresentaram taxa de utilização inferior aos demais grupos. O diâmetro, perímetro e área do CL foram semelhantes (P > 0,1) em todos os grupos. No entanto, o grupo hCG resultou em CL com melhor escore de vascularização (P = 0,04), maior fluxo sanguíneo central (P = 0,03) e tendência a maior fluxo sanguíneo periférico (P = 0,08). As taxas de concepção (32,00% hCG vs. 35,10% eCG; P = 0,46) e prenhez (24,69% hCG vs. 29,61% eCG; P = 0,20) foram semelhantes entre os grupos hCG e eCG. No entanto, uma interação entre o tratamento gonadotrópico e a categoria revelou menor concepção (P = 0,01) e taxas de prenhez (P = 0,001) em receptoras nulíparas tratadas com hCG. O tratamento com hCG resultou em maior intensidade de expressão de estro e CL com maior escore de vascularização, o que determinou taxas de utilização, concepção e prenhez semelhantes aos protocolos convencionais com eCG. No entanto, receptoras nulíparas tratadas com hCG exibiram uma taxa reprodutiva geral mais baixa.

Palavras-chave: tratamento gonadotrófico, hCG, eCG, transferência de embriões, avaliação Doppler.

ROSSIGNOLO, Eduardo Antônio de Almeida. 2023. **Evaluation of hCG as gonadotropic support to timed embryo transfer protocol in beef cattle** 50 fls. Dissertation (Master in Veterinary Clinics) – State University of Londrina, Londrina, 2023.

ABSTRACT

This study compared the reproductive performance of embryo recipients treated with a timed embryo transfer (TET) protocol using human chorionic gonadotropin (hCG) or equine chorionic gonadotropin (eCG). On a random day of the estrous cycle (Day -10) *Bos taurus indicus* recipients (n=341; 194 nulliparous and 147 multiparous cows) with a body condition score between 3.0 and 4.0, were submitted to the TET protocol consisting of an intramuscular (i.m.) injection of 2.0 mg estradiol benzoate (EB) and the insertion of intravaginal progesterone (P4) device that remained until Day -2.5. On the same day (-2.5), the recipients received i.m. 150 mg D-cloprostenol and 1 mg estradiol cypionate and were randomly divided into two groups: the eCG group (n = 179), in which females received i.m. 300 IU eCG and the hCG group (n=162), in which females received 150 IU hCG. Then, estrus intensity and the diameter of the dominant follicle (DF) were monitored on D0 and the quality of the corpus luteum (CL) (B mode and color Doppler) was assessed on D7 to select recipients eligible for receiving the transfer of an embryo produced *in vitro*. Pregnancy diagnosis was assessed 23 days after the transfer. Continuous data were analyzed by ANOVA using a mixed-effects model and Tukey's test. The rates were analyzed using a logistic regression model. The diameter of the DF on day 0 of the TET protocol was influenced by the interaction between gonadotropic treatment and category (P=0.01), and nulliparous recipients treated with hCG had the smallest diameter. Treatment with hCG and eCG resulted in a high rate of estrus expression; however, the proportion of females with a high-intensity of estrus was higher in the hCG group (79.84 vs. 68.61%, respectively; P = 0.03). The utilization rate (recipients with CL) showed a tendency (P=0.06) to be influenced by the interaction between gonadotropic treatment and category, wherein nulliparous recipients treated with hCG exhibited a lower utilization rate than the other groups. The diameter, perimeter, and area of the CL were similar (P>0.1) in all groups. However, the hCG group resulted in CL with a better Doppler evaluation score (P=0.04), central blood flow (P=0.03), and tendency towards greater peripheral blood flow (P=0.08). The rates of conception (32.00% hCG *vs.* 35.10% eCG; P=0.46) and pregnancy (24.69% hCG *vs.* 29.61% eCG; P=0.20) were similar between the hCG and eCG groups. However, an interaction between the gonadotropic treatment and category revealed lower conception (P = 0.01) and pregnancy rates (P=0.001) in nulliparous recipients treated with hCG. Treatment with hCG resulted in a greater intensity of estrus expression and CL with a higher Doppler score, which determined rates of utilization, conception, and pregnancy similar to conventional protocols using eCG. However, nulliparous recipients treated with hCG exhibited a lower overall reproductive rate.

Key words: gonadotropic treatment, hCG, eCG, embryo transfer, Doppler evaluation

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LISTA DE ABREVIATURAS E SIGLAS

CL	Corpo lúteo
ECC	Escore de condição corporal
eCG	Gonadotrofina coriônica equina
E2	Estradiol
GnRH	Hormônio liberador de gonadotrofinas
hCG	Gonadotrofina coriônica humana
IATF	Inseminação artificial em tempo fixo
TETF	Transferência de embrião em tempo fixo
IVD	Embrião *in vivo*
IVP	Embrião *in vitro*
PGF2α	Prostaglandina f 2alfa
P4	Progesterona

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1. INTRODUÇÃO

As biotécnicas reprodutivas tem contribuído fortemente para o incremento de produtividade e eficiência dentro da cadeia de produção pecuária, de acordo com BARUSELLI (2023) o mercado de inseminação artificial em tempo fixo (IATF) e transferência de embrião em tempo fixo (TETF) tem crescimento vertiginoso desde 2002, com uma taxa de crescimento composta de 31,5%, muito embora o mercado neste último ano, apresentou uma retração de aproximadamente 6% em número de protocolos comercializados e aproximadamente 10% no número de doses comercializadas. No entanto a proporção de uso das biotécnicas passou de 93% para quase 98% em relação ao último boletim.

Durante as últimas duas décadas diversos estudos foram conduzidos para melhor compreensão da fisiologia reprodutiva, além da possibilidade de associações de fármacos em diferentes momentos (BARUSELLI, 2018; MAPLETOFT et al., 2022), bem como o desenvolvimento de estratégias, drogas e a dinâmica de uso nas fazendas. Basicamente o protocolo hormonal é baseado em estradiol (E2) e progesterona (P4) (BARUSELLI et al., 2004; SALES et al., 2012; BOSOLASCO et al., 2021), ou então P4 e fator liberador de gonadotrofina (GnRH) (RODRIGUES et al, 2019; MADUREIRA, et al, 2020) e este associado ao uso de prostaglandina ($PGF2\alpha$) ao término do protocolo (LAMB et al., 2010; MADUREIRA et al., 2020).

Buscando a otimização de lotes e elevar o sucesso da TETF, protocolos hormonais similares ao da IATF tem sido empregado nos programas de TETF, tornando-se interessante seu emprego em grandes volumes (BÓ et al., 2011; MAPLETOFT et al., 2022). Essa manipulação hormonal proporcionou, assim como para a IATF, a dispensa de observação de estro, melhor gestão do lote de receptora, otimização no uso das receptoras dispensando a necessidade de enormes planteis dentro das centrais de transferência de embrião (BÓ et al., 2011).

A utilização de gonadotrofinas em protocolos IATF, contribuiu para o aumento da resposta das fêmeas submetidas à esse protocolo (BARUSELLI et al., 2004; BARREIROS et al., 2014), por ter sua função relacionada ao melhor desenvolvimento folicular contribuindo por um aumento na taxa de ovulação destas fêmeas (SÁ FILHO et al., 2010; PRATA et al., 2018). A gonadotrofina coriônica equina, secretada pelos cálices endometriais de éguas prenhes, de 40 a 100 dias de gestação (SENGER, 2012), tem sido a gonadotrofina de maior emprego, por sua função majoritária de hormônio folículo estimulante (FSH), acompanhada de hormônio luteinizante (LH), tem seu efeito marcante em animais com baixa condição de escore corporal (ECC), além de fêmeas lactantes e novilhas pré púberes (SÁ FILHO et al., 2010; BARREIROS et al., 2014; PRATA et al., 2018). A outra gonadotrofina, trata-se da gonadotrofina coriônica humana (hCG), produzida pelo trofoblasto humano e excretada em grandes quantidades na urina de mulheres gestantes (DE MEDEIROS et al., 2008). Em bovinos a hCG se liga aos receptores de LH da célula da granulosa e tem efeito na indução da ovulação (IRLAND et al., 1982,1983; BUTTREY et al., 2010; KESKIN et al., 2010). Além disso, por ser uma molécula grande, alto peso molecular (~38000 Da), apresenta grande período de ação, até 30 horas após o tratamento (GIORDANO et al.,2013).

A ultrassonografia em modo B, do aparelho reprodutivo, teve seu marco temporal iniciado em 1983, inicialmente em equinos e no ano seguinte em bovinos, e a partir disso, a fisiologia ovariana passou a ser estudada para o melhor entendimento proporcionando assim intervenções e averiguação do que os fármacos causavam na dinâmica ovariana (GUINTER, 2007). Concomitante a técnica em modo B, a ultrassonografia, sendo ela: color, espectral e power doppler passaram a ser empregadas em exames reprodutivos com certa frequência (PUGLIESI et al., 2017), e desta forma proporcionou exames mais sensíveis a fim de promover respostas mais precoces, acuradas e contribuir para a tomada de decisões em tempo real.

Este estudo pretende comparar o desempenho reprodutivo de receptoras de embriões tratadas com um protocolo de TETF usando hCG ou eCG.

2. REVISÃO DE LITERATURA

2.1 Transferência de embrião em tempo fixo

A TETF, como é popularmente chamada, é a biotécnica reprodutiva que consiste na transferência dos embriões, em suma produzidos *in vitro*, e transferidos em fêmeas sincronizadas com o protocolo de IATF (BARUSELLI et al., 2009; BÓ et. al., 2011a), e desta forma, assim como na IATF, dispensa a detecção do estro e contribui para minimizar falhas na detecção, e com o adicional de trabalhar fêmeas em anestro pós parto, desta forma aumentando as fêmeas aptas a serem utilizadas dentro das centrais de transferência de embrião (BARUSELLI et.al.,2011).

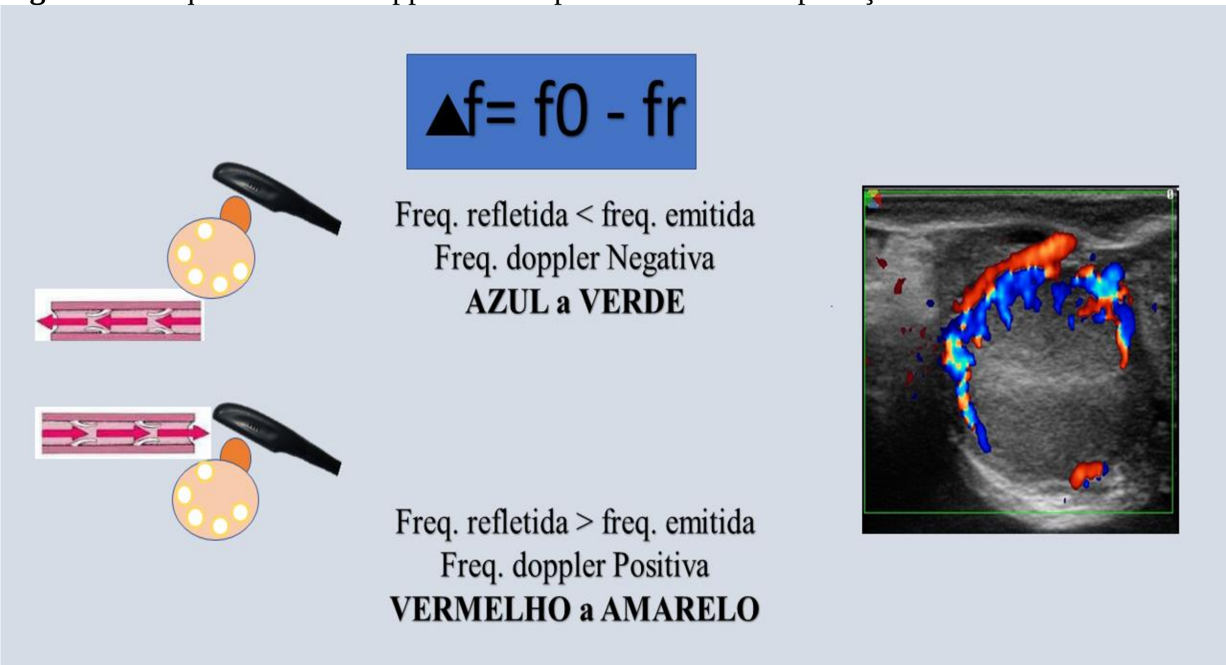
Nos últimos 15 anos, TETF vem passando por diversas transformações em virtude do avanço tecnológico em termo de equipamentos, seja laboratorial, ou em equipamentos de imagem e sem dúvidas na mudança de chave na técnica de produção. Por volta de 2012 a produção *in vivo* (IVD) apresentou uma tendência de queda e a produção *in vitro* (IVP) apresentou a tendência de alta, e de 2012 a 2016 as duas técnicas andaram lado a lado, embora até 2016, a liderança ainda foi da IVD, e que a partir de 2017, principalmente aos avanços na criopreservação dos embriões oriundos de IVP (SANCHES et.al., 2016), esta assume a liderança em quantidade de embriões produzidos (VIANA, J.H.M. 2022).

2.2 Ultrassonografia modo B e Doppler

O efeito doppler foi descrito inicialmente em 1842 por Christian Johann Doppler, para diagnóstico de gestação em cadelas (SZATMÁRI et al., 2001; NOGUEIRA et al., 2017; PUGLIESI et al., 2017). O ***color doppler*** é uma das três variações do modo doppler, que caracteriza a variação dos ecos emitidos e recebido atribuindo-lhes cor, geralmente azul ou vermelho como demonstrado na figura 1.

A intensidade da cor é diretamente relacionada com a velocidade do fluxo sanguíneo (NOGUEIRA et al., 2017). Ainda são possíveis outras duas avaliações com modo doppler, são elas: modo ***doppler espectral***, em que o resultado é obtido em um gráfico de velocidade em função do tempo utilizado para avaliações cardíacas e o ***power doppler***, método quantitativo do color doppler, que a escala de cores varia em função da quantidade de células presentes em determinado capilar ou vaso.

Figura 1- Frequência color doppler em corpo lúteo e sua interpretação



(BOLLWEIN et al., 2013; PUGLIESI et al., 2017; BALARO et al., 2017 – Adaptado)

Em equipamentos com a função color doppler, as diferenças de frequência são codificadas na forma de sinais coloridos na tela do equipamento sobre uma imagem em modo B (escala de cinza) convencional. Diferenças positivas (fluxo sanguíneo em sentido ao transdutor) e negativas (fluxo sanguíneo em sentido contrário ao transdutor) são indicadas por cores diferentes, que são geralmente em tons de vermelho a amarelo e azul a verde.

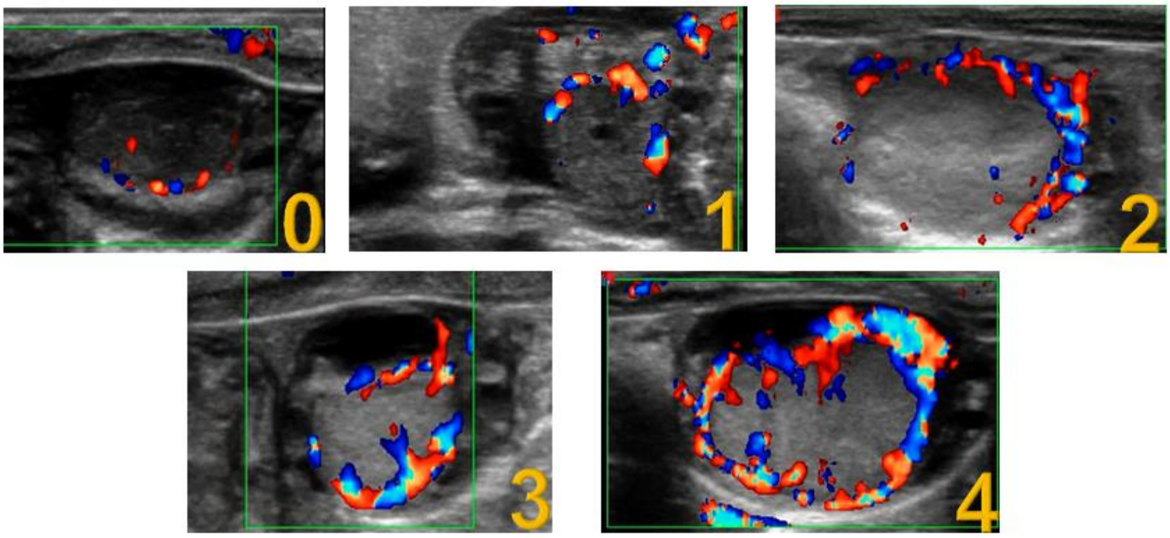
Na reprodução o **color doppler** tem sido utilizado para avaliação da função luteal de forma menos invasiva, de modo que o fluxo sanguíneo presente no corpo lúteo (CL) é refletido na capacidade de função lútea. Sabidamente a função luteal apresenta alta relação com a função angiogênica do CL, assim é possível a realização de uma varredura do CL e sua correção com os níveis de progesterona (P4) circulante (BOLLWEIN et al., 2013). Avaliações com esse intuito podem fornecer valiosas informações quanto a fisiologia reprodutiva destes animais (BOLLWEIN et al., 2013; BALARO et al., 2017).

Diversos pesquisadores têm se dedicado na avaliação da função luteal de bovinos com a técnica de color doppler e assim surgiu a necessidade de uma metodologia altamente eficiente e aplicável para a pesquisa, mas principalmente reproduzível no campo. Inicialmente as imagens eram realizadas no campo e salvas em um dispositivo que por meio de um software eram lidas e quantificadas o número de pontos coloridos (pixels). Esse processo tornava o sistema ineficiente quanto a tomada de decisões em qualquer que fosse a situação, seja para diagnóstico preditivo de gestação ou avaliação de funcionalidade do CL em receptoras de TETF (VIANA et al., 2013). Buscando uma alternativa para a utilização da informação obtida pelo color doppler em tempo real, criou-se uma escala de escore de vascularização (GHETTI et al., 2012). Essa metodologia mensura o sinal doppler de acordo com sua distribuição no CL avaliado (Figura 2), sendo necessário a atenção e o preparo do examinador para uma aferição mais adequada e padronizada. Assim surgiu o modelo de escore, modelo de avaliação subjetiva que apresenta alta correlação com a avaliação objetiva de fluxo sanguíneo (PUGLIESI et al., 2017).

Quando se trata de estruturas ovarianas apresentando baixo sinais de vascularização, esta análise pode ser prejudicada, enquanto quando há sinais doppler em abundância, a análise pode ser facilmente realizada, não restando dúvidas (VIANA et al., 2013). O escore de vascularização quantifica de forma subjetiva a proporção de irrigação sanguínea do CL. Vacas não gestantes, com escore de 0 e 1 possuem irrigação do CL < 25% e área do CL < 2 cm2, enquanto as vacas com escore de 2, 3 e 4 são dadas como gestantes, apresentando escore de irrigação ≥25% e área > 2 cm² (PUGLIESI et al., 2017).

Embora a ultrassonografia color doppler venha se consolidando como uma ferramenta atual, não podemos deixar de abordar postos-chave da técnica como a especificidade e sensibilidade na predição de gestação aos 20 e 22 dias após inseminação. Dados atuais mostram uma grande sensibilidade, isto é, a capacidade de apontar os animais gestantes, em torno de 100% (GUIMARÃES et al., 2015; PUGLIESI et al., 2014) e especificidade moderada de 79,3 (GUIMARÃES et al., 2015) além de apresentar uma morte embrionária maior que na avaliação aos 30 dias 4,1% p=0,21 (SÁ FILHO et al., 2014).

Figura 2 - Imagens ultrassonográficas de ovário bovino apresentando corpo lúteo. Os painéis 0 a 4 indicam, respectivamente, os escores 0, 1, 2, 3 e 4 para perfusão sanguínea periférica e central no tecido luteal dos animais avaliados (limite de detecção: 0,05 m/s). Painéis 0 e 1: vacas não gestantes; Painéis 2 a 4: vacas gestantes.



(PUGLIESI, 2017; SIQUEIRA, 2012 – Adaptado)

2.3 Gonadotrofina coriônica equina (eCG)

A gonadotrofina coriônica equina (eCG), hormônio glicoproteico, possui subunidades α e β, similares ao LH e FSH, embora apresentem estruturalmente maior peso em virtude de maior concentração de carboidratos, especialmente no ácido ciálico, o que confere a esta molécula hormonal, longa meia vida (HAFEZ E HAFEZ, 2004; SENGER, 2012).

O eCG é produzido, por estruturas endometriais especializadas denominadas cálices endometriais de éguas gestantes a partir dos 40 dias de gestação perdurando sua liberação em até 100 dias (SENGER, 2012). Nestes animais a função do eCG é produzir corpos lúteos acessórios, que produzem P4 para a manutenção da gestação (HAFEZ E HAFEZ, 2004; SENGER, 2012)

Na TETF, para atender a necessidade de eliminar-se a observação de cio, melhorar a gestão do rebanho de receptoras e aumentar a taxa de aproveitamento dentro das centrais, o eCG surge como opção para o incremento nos resultados tanto de sincronização quanto de maior prenhez nos grupos tratados com este hormônio (NASER et al, 2004; BÓ et al, 2011)

Haja vista o benefício que esta gonadotrofina trouxe para os protocolos de sincronização e indução da ovulação em receptoras de embrião, diversos ajustes foram feitos ao longo dos anos dentro desta evolução das biotecnicas no Brasil, hoje consolida-se protocolos a base de E2/P4 e obrigatoriamente com utilização do eCG como a gonadotrofina de primeira escolha e assim sendo a demanda por este hormônio cresceu (BARUSELLI, 2023). Por este fato, e levando em consideração o indivíduo produtor, a fêmea gestante equina, há a necessidade de buscar opções para a sua substituição, sem prejuízos nos resultados.

2.4 Gonadotrofina coriônica humana (hCG)

A gonadotrofina coriônica humana, assim como o eCG, também se trata de uma glicoproteína, com duas subunidades, α e β, com peso molecular de ~38000 Da. Sua subunidade α, composta por 92 aminoácidos e duas cadeias de carboidratos. No hCG, a subunidade α, tem similaridade com o LH em humanos, suínos, ovinos e bovinos. Majoritariamente o hCG é luteinizante e luteotrófico, com baixa atividade de FSH. É produzido pelo trofoblasto na placenta, com alta disponibilidade no sangue e secretado em grande quantidade na urina (HAFEZ E HAFEZ, 2004; SENGER, 2012; PRATA, et al. 2018).

O hCG é utilizado de rotina na reprodução equina, principalmente com indutor de ovulação (BARBACINI et al, 2000). Em bovinos este hormônio tem sua utilização principal, também, como agente indutor da ovulação (SENGER, 2012), contudo atualmente, em programas de IATF, o cipionato de estradiol (CE) assumiu esta função (BARUSELLI, 2004).

Em protocolos de IATF, o hCG foi testado como substituo ao eCG (PRATA et al, 2018) apresentando como opção para promover a ovulação do folículo dominante, no entanto, as dosagens necessárias para este desfecho foram responsáveis por antecipar a ovulação e desta forma apresentaram resultados insatisfatórios.

Diante deste cenário, sabendo do seu alto peso molecular, apresenta meia vida de aproximadamente 30 horas, é então, candidato a promover o crescimento folicular, resultando em ovulação e formação de corpo lúteo (PRATA et al, 2018), este fator primordial para se realizar a transferência de embrião (BÓ et al, 2011).

REFERÊNCIAS

BARUSELLI, P.S. Com desaceleração de 5% em 2022, mercado da IATF registra primeiro recuo em 20 anos. Boletim Eletrônico do Departamento de Reprodução Animal/FMVZ/USP, 7ª ed., 2023. Acesso http://vra.fmvz.usp.br/boletim-eletronico-vra/

BARUSELLI P, SALES J, SALA R, VIEIRA L, SÁ FILHO M. History, evolution and perspectives of timed artificial insemination programs in Brazil. Animal Reproduction. V. 9, p. 139-52, 2018.

MAPLETOFT, R.J.; BÓ, G.A.; BARUSELLI P.S.; MENCHACA, A.; SARTORI, R. Evolution of knowledge on ovarian physiology and its contribution to the widespread application of reproductive biotechnologies in South American cattle. Animal Reproduction. V. 3; 15 (Suppl 1) p. 1003-1014, 2018

BARUSELLI, P.S.; REIS, E.L.; MARQUES, M.O.; NASSER, L.F.; BÓ, G.A. The use of hormonal treatments to improve reproductive performance of anestrous beef cattle in tropical climates. Animal Reproduction Science, v. 82–83, p. 479-486, 2004.

SALES, J.N.; CARVALHO, J.B.; CREPALDI, G.A.; CIPRIANO, R.S.; JACOMINI, J.O.; MAIO, J.R.; SOUZA, J.C.; NOGUEIRA, G.P.; BARUSELLI, P.S. Effects of two estradiol esters (benzoate and cypionate) on the induction of synchronized ovulations in Bos indicus cows submitted to a timed artificial insemination protocol. Theriogenology, v. 78, p. 510-6, 2012.

BOSOLASCO, D.; NUÑEZ-OLIVERA, R.; DE BRUN, V..; MEIKLE, A.; MENCHACA, A. Estradiol cypionate administered at the end of a progesterone-based protocol for FTAI induces ovulation and improves postovulatory luteal function and uterine environment in anestrous beef cows . Theriogenology, v.162, p. 74-83, 2021.

MADUREIRA, G.; CONSENTINI, C.E.; MOTTA, J.C.; DRUM, J.N.; PRATA, A.B.; MONTEIRO, P.L.J. JR.; MELO, L.F.; GONÇALVES, J.R.S.; WILTBANK, M.C.; SARTORI, R. Progesterone-based timed AI protocols for Bos indicus cattle II: reproductive outcomes of either EB or GnRH-type protocol, using or not GnRH at AI. Theriogenology, v.145, p.86-93, 2020.

RODRIGUES, W.B.; SILVA, A.S.; SILVA, J.C.B.; ANACHE, N.A.; SILVA, K.C.; CARDOSO C.J.T.; GARCIA, W.R.; SUTOVSKY, P.; NOGUEIRA, E. Timed artificial insemination plus heat II: gonadorelin injection in cows with low estrus expression scores increased pregnancy in progesterone/estradiol-based protocol. Animal, v. 13, p. 2313-8, 2019.
LAMB, G.C.; DAHLEN, C.R.; LARSON, J.E.; MARQUEZINI, G.; STEVENSON, J.S.; Control of the estrous cycle to improve fertility for fixed-time artificial insemination in beef cattle: a review. Jornal Animal Science, v. 88, p. E181-92, 2010.

BÓ, G.A.; PERES, L.C.; CUTAIA, L.E.; PINCINATO, D.; BARUSELLI, P.S.; MAPLETOFT, R.J. Treatments for the synchronisation of bovine recipients for fixed-time embryo transfer and improvement of pregnancy rates. Reproduction Fertility Development, v. 24, p. 272-7, 2011.

BARREIROS, T.R.; BLASCHI, W.; SANTOS, G.M.; MOROTTI, F.; ANDRADE, E.R.; BARUSELLI, P.S.; SENEDA, M.M. Dynamics of follicular growth and progesterone concentrations in cyclic and anestrous suckling Nelore cows (Bos indicus) treated with progesterone, equine chorionic gonadotropin, or temporary calf removal. Theriogenology, v. 81, p. 651-6, 2014.

SA FILHO, M.F.; CRESPILHO, A.M.; SANTOS, J.E.; PERRY, G.A.; BARUSELLI, P.S. Ovarian follicle diameter at timed insemination and estrous response influence likelihood of ovulation and pregnancy after estrous synchronization with progesterone or progestin-based protocols in suckled Bos indicus cows. Animal Reproduction Science, v.120, p. 23-30, 2010.

PRATA, A.B.; DRUM, J.N.; MELO, L.F.; ARAUJO, E.R.; SARTORI, R. Effect of different chorionic gonadotropins on final growth of the dominant follicle in bos indicus cows. Theriogenology, v. 111, p. 52-55, 2018.
SENGER, P.L. Pathways to pregnancy and parturition, Redmond, OR, Current Conceptions Inc, 3ed., p. 381, 2012

DE MEDEIROS, S.F.; NORMAN, R.J. Human choriogonadotrophin protein core and sugar 247 branches heterogeneity: basic and clinical insights. Human Reproduction Update, v. 15, p. 69- 95, 2008.

IRELAND, J.J.; ROCHE, J.F. Development of antral follicles in cattle after prostaglandin-induced luteolysis: Changes in serum hormones, steroids in follicular fluid, and gonadotropin receptors. Endocrinology, v. 111, p. 2077-86, 1982.

BUTTREY, B.S.; BURNS, M.G.; STEVENSON, J.S. Ovulation and pregnancy outcomes in response to human chorionic gonadotropin before resynchronized ovulation in dairy cattle. Theriogenology, v. 73, p. 449-59, 2010.

KESKIN, A.; YILMAZBAS-MECITOGLU, G.; GUMEN, A.; KARAKAYA, E.; DARICI, R.; OKUT, H. Effect of hCG vs. GnRH at the beginning of the Ovsynch on first ovulation and conception rates in cyclic lactating dairy cows. Theriogenology, v. 74, p. 602–7, 2010.

GIORDANO, J.O.; WILTBANK, M.C.; GUENTHER, J.N.; ARES, M.S.; LOPES, G. JR.; HERLIHY, M.M.; FRICKE, P.M. Effect of presynchronization with human chorionic gonadotropin or gonadotropin-releasing hormone 7 days before resynchronization of ovulation on fertility in lactating dairy cows. Jornal Dairy Science, v. 95, p. 5612-25, 2012.

GINTHER, O. J. Ultrasonic imaging and animal reproduction: color-Doppler ultrasonography. Cross Plains, WI: Equiservices Publishing, p. 247, 2007.

PUGLIESI, G.; REZENDE, R. G.; SILVA, J. C. B.; LOPES, E.; NISHIMURA, T. K.; BARUSELLI, P. S.; MADUREIRA, E. H.; BINELLI, M. Uso da ultrassonografia Doppler em programas de IATF e TETF em bovinos. Revista Brasileira Reprodução Animal, v. 41, n. 1, p. 140–150, 2017.

BARUSELLI, P. S.; ROBERTA, A. E.; FERREIRA, A. M.; SÁ FILHO, A. M. F.; NASSER, L. F. T. B.; RODRIGUES, C. A. C.; BÓ, G. A. Bovine embryo transfer recipient synchronisation and management in tropical environments. Reproduction, Fertility and Development, v. 22, n. 1, p. 67-74, 2009

BÓ, G. A.; PERES, L. C. A.; CUTAIA, A. L. E.; PINCINATO, D.; BARUSELLI, P. S.; MAPLETOFT, R. J.; Treatments for the synchronisation of bovine recipients for fixed-time embryo transfer and improvement of pregnancy rates. Reproduction, Fertility and Development, v. 24, n. 1, p. 272-277, 2011.

BARUSELLI, P. S.; FERREIRA, R.M. ; SALES, J.N.S. ; GIMENES, L. U.; SÁ FILHO, M. F.; MARTINS, C. M.; RODRIGUES, C. A.; BÓ, G. A. Timed embryo transfer programs for management of donor and recipient cattle. Theriogenology, v. 76, p. 1583-1593, 2011.

SANCHES, B.V.; LUNARDELLI, P.A.; TANNURA, J.H.; CARDOSO, B.L.; PEREIRA, M.H.; GAITKOSKI, D.; BASSO, A.C.; ARNOLD, D.R.; SENEDA, M.M. A new direct transfer protocol for cryopreserved IVF embryos. Theriogenology, v. 1, n. 85(6), p. 1147-51, 2016.

VIANA, J. H. M. Statistics of embryo production and transfer in domestic farm animals, Embryo Technology Newsletter, v. 40, n.4, 2022.

SZATMÁRI, V.; SÓTONYI, P.; VÖRÖS, K.; Normal duplex Doppler waveforms of major abdominal blood vessels in dogs: A review. Veterinary Radiologic Ultrasound v. 42, p. 93–107, 2001.

NOGUEIRA, É., SILVA, J.C.B., NICACIO, A.C., MINGOTI, G.Z., Ultrassonografia na reprodução e avaliação de carcaças em bovinos, Brasília – DF, 1 ed., Embrapa, , 2017.

PUGLIESI, G.; REZENDE, R.G.; SILVA, J.C.B. DA; LOPES, E.; NISHIMURA, T.K.; BARUSELLI, P.S.; MADUREIRA, E.H.; BINELLI, M. Uso da ultrassonografia Doppler em programas de IATF e TETF em bovinos. Revista Brasileirade Reprodução Animal, v. 41, p. 140–150, 2017.

BOLLWEIN, H.; LÜTTGENAU, J.; HERZOG, K. Bovine luteal blood flow: basic mechanism and clinical relevance. Reproduction, Fertility and Development, v. 25, n. 1, p. 71–79, 2013.

BALARO, M.F.A.; SANTOS, A.S.; MOURA, L.F.G.M.; FONSECA, J.F.; BRANDÃO, F.Z.; Luteal dynamic and functionality assessment in dairy goats by luteal blood flow, luteal biometry, and hormonal assay. Theriogenology, v. 95, p. 118–126, 2017.

VIANA, J.H.; URASHIRO, E.K.; SIQUEIRA, L.G.; GHETTI, A.; AREAS, V.; GUIMARÃES, C.R.; PALHÃO, M.; CAMARGO, L.S.; FERNANDES, C.; Doppler ultrasonography as a tool for ovarian management. Animal Reproduction, v. 10, p. 215–222, 2013.

GHETTI, A. M.; SIQUEIRA, L. G. B.; AREAS, V. S.; RASHIRO, E. K. N.; FERNANDES, C. A. C.; PALHAO, M. P.; VIANA, J. H. M. Comparison of objective methods for evaluating the vascular pattern of the preovulatory follicle using color doppler. Animal Reproduction 9, 657 2012 (abstract).

GUIMARÃES, C. R. B.; OLIVEIRA, M. E.; ROSSI, J. R.; FERNANDES, C. A. C.; VIANA, J. H. M.; PALHAO, M. P. Corpus luteum blood flow evaluation on Day 21 to improve the management of embryo recipient herds. Theriogenology, v. 84, n. 2, p. 237–241, 2015.

PUGLIESI, G.; MIAGAWA, B. T.; PAIVA, Y. N.; FRANÇA, M. R.; SILVA, L. A.; BINELLI, M. Conceptus-induced changes in the gene expression of blood immune cells and the ultrasound-accessed luteal function in beef cattle: how early can we detect pregnancy? Biology of Reproduction, v. 91, n. 4, p. 1–12, 2014.

SÁ FILHO, M. F.; MARQUES, M. O.; GIROTTO, R.; SANTOS, F. A.; SALA, R. V.; BARBUIO, J. P.; BARUSELLI, P. S. Resynchronization with unknown pregnancy status using progestin-based timed artificial insemination protocol in beef cattle. Theriogenology, v. 81, n. 2, p. 284–290, 2014.

HAFEZ, B., HAFEZ, E. S. E. Anatomia da reprodução feminina. In: HAFEZ, E. S. E. Reprodução animal. São Paulo: Manole, 7 ed., p. 261-278, 2004.

NASSER, L.F.; REISA, E.L.; OLIVEIRA, M.A.; BÓ, G.A.; BARUSELLI, P.S. Comparison of four synchronization protocols for fixed-time bovine embryo transfer in Bos indicus Bos taurus recipientes. Theriogenology, v. 62, p. 1577–1584, 2004.

3. HIPÓTESE

A substituição de eCG por hCG, promove a mesma taxa de prenhez e a mesma taxa de concepção em protocolos de TETF em gado de corte. Além de aumentar a taxa de aproveitamento das receptoras e tornar o processo de TETF mais eficiente.

4. OBJETIVOS

4.1 Objetivo geral

Avaliar o efeito da substituição de eCG por hCG em receptoras nelore nulíparas e multíparas, nelore e meio sangue angus-nelore.

4.2 Objetivos específicos

- Estabelecer a melhor categoria a ser realizada essa substituição

- Reduzir o custo de sincronização ao efetuar a substituição da gonadotrofina sem comprometer Taxa de prenhez e Taxa de concepção.

5. ARTIGO PUBLICADO

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1. Introduction

Ovulation synchronization protocols have evolved greatly over the last two decades because of increasing studies that have improved our understanding of reproductive physiology [1, 2], the development of new drugs, and efficient combination hormonal treatments [3, 4] that favor effective synchrony in follicular emergence, control of the progesterone (P4) phase, and ovulation induction [5]. These hormonal treatments often combine the use of P4 and estradiol [6, 7] or P4 and gonadotropin-releasing hormone (GnRH) [8, 9], associated with the use of prostaglandins at the end of the hormonal protocol [10]. Such hormonal combinations have been shown to be highly effective in synchronizing large herds by optimizing management and increasing fertility rates in both dairy and beef cattle [11, 12], and from different females categories [13] due to effective control in the synchronization of estrus and ovulation [14, 15].

To improve fertility in embryo transfer programs, ovulation synchronization protocols for timed artificial insemination (TAI) have been tested for the recipients synchronization [16-18]. These hormonal treatments optimized synchronization management, excluded the need for estrus observation, allowed transfer in a fixed time, and reduced the need to maintain large recipient herds, increasing the rates of recipients utilization and pregnancy in the programs [18, 19]. However, under the management conditions of most tropical herds, postpartum anestrus is a major fertility limiter in both TAI and timed embryo transfer (TET) programs. This is due to the low amount of luteinizing hormone (LH) stored in the pituitary, which limits the restoration of ovarian activity during the postpartum period [20] and that is intensified under inadequate nutritional conditions or suckling frequency [21]. Therefore, most timed hormonal protocols use equine chorionic gonadotropin (eCG) as an additional gonadotropic stimulus at the end of P4 treatment to increase follicular growth, ovulation rate, corpus luteum (CL), luteotrophic activity and improve conception [22-24].

eCG is a drug with a long half-life (up to 3 days), produced in the endometrial calyxes of mares with 40 to 130 days of gestation [25]; in cows, eCG binds the follicle-stimulating hormone (FSH) and LH receptors of the dominant follicle, stimulating its growth and increasing ovulation rate [26], as well as, contributing to greater luteotrophic activity [25, 27]. Administration of eCG before ovulation elevates plasma P4 levels in the subsequent estrous cycle due to its stimulatory effect on the theca and granulosa cells of the preovulatory follicle [25, 28], thus favoring ovulation of a larger follicle, giving rise to a larger corpus luteum (CL) [27] and increasing conception [17, 22, 23]. In general, 300 to 400 IU of eCG is used between the 5th and 8th days of the estradiol/P4-based synchronization protocol. Overall, 75-85% of recipients treated with this protocol receive an embryo, P4 concentrations are high at the time of embryo transfer, and conception generally exceeds 50% when both embryos and recipients are of high quality [18, 29], which is a well-established pharmacological strategy for TAI and TET programs.

However, the use of eCG presents great challenges, particularly in the purification of eCG produced by pregnant mares only between 40 and 130 days of gestation [25, 30]. The extraction and purification process is complex and costly, therefore, eCG is a drug with relatively high costs. In addition to not being free of contaminants, it can vary in products batches, as well as raises concerns about animal welfare [31, 32]. Human chorionic gonadotropin (hCG) represents an interesting alternative gonadotropic stimulus as it can bind to follicular FSH and LH receptors. hCG has a biological activity of approximately 30 h [33], is extracted from the urine of pregnant women [34], and is more related to the effect of LH than FSH [26, 35-37].

The most common use of hCG has been as a substitute for GnRH in TAI protocols [38, 39] or its isolated use 4-15 days after insemination to induce accessory CL formation and increase P4 concentration [40-42]. However, the effects of hCG during protocol of TAI are dose-dependent [43]. High doses (300 to 3.000 IU) have highlighted its role as an ovulation inducer [38, 43], and low doses (150 to 200 IU) have the effect of stimulating follicular growth [43, 44]. Studies have been conducted to replace eCG with hCG in the TAI protocol [26, 43] and have shown to result in similar reproductive indices with adjustments in the time of insemination [44]. However, to the best of our knowledge, no study has evaluated the use of hCG to replace eCG in TET protocols. Certainly, the use of hCG in TET programs seems to be very strategic and much less critical because it is no need for such a fine adjustment at the time of ovulation, as required in TAI programs. Therefore, we hypothesized that hCG stimulates follicular growth, induces the formation of higher quality CL and results in an acceptable fertility rate compared to eCG, being a viable alternative for use in the TET protocol. To test this hypothesis, we compare the reproductive performance of multiparous and nulliparous embryo recipients submitted to TET protocols employing hCG or eCG.

2. Materials and methods

2.1. Location, animals, and management

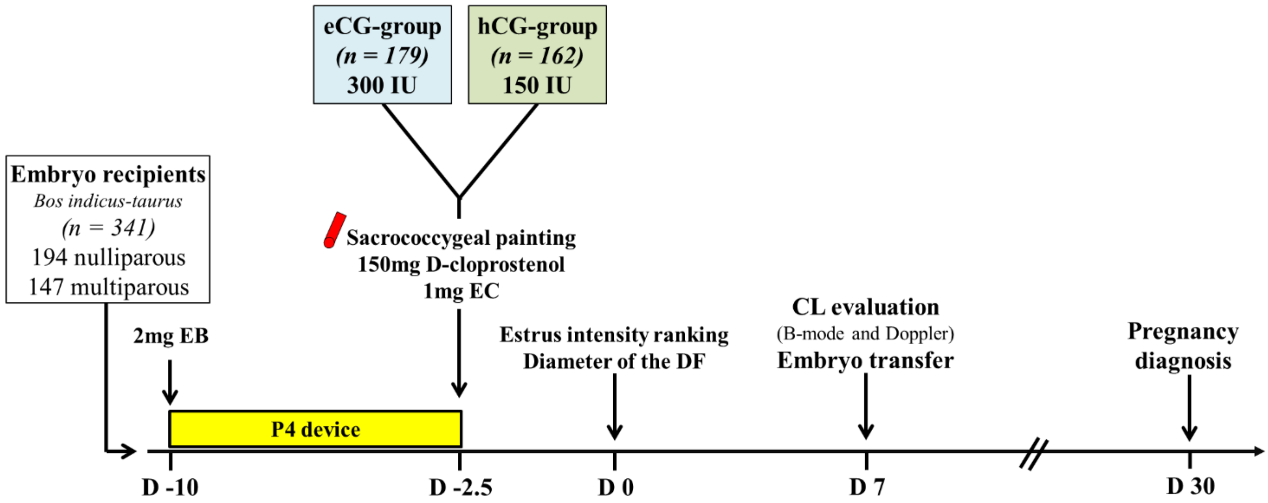
The present study was performed during the typical beef cattle breeding period in South America at the Biotec-Unicesumar experimental farm located at -23°34′18″ S and -51°87′52″ W under tropical climate conditions. The climate is classified as Cfb (wet subtropical climate), with an average annual temperature of 22°C and precipitation of 2.913 mm.

For this study, we used potential *indicus-taurus* (Nelore (*Bos indicus*) × Angus (*Bos taurus*)) embryo recipients ($n = 341$), 194 nulliparous/heifers, and 147 multiparous cows with 30 to 60 days postpartum, a body condition score (BCS) between 3.0 and 4.0 (scale of 1 to 5) and kept in a continuous grazing system with adjustment for adequate stocking rate and *ad libitum* access to mineral salt and water. In addition, both categories of females underwent a vaccination protocol against reproductive diseases (Cattle Master Gold®, Zoetis, Sao Paulo, Brazil) with a booster dose 30 days later.

2.2. Timed embryo transfer protocol and experimental design

Previously, animals were evaluated by gynecological examination (vulvar inspection, palpation and transrectal ultrasound of the cervix, uterus and ovary) for the selection of females for the reproductive program. These recipients were part of a commercial TET program. On a random day of the estrous cycle (day -10), the females were intramuscularly (i.m.) injected with 2.0 mg estradiol benzoate (Gonadiol®; Zoetis, Sao Paulo, Brazil) and an intravaginal progesterone (P4) device (CIDR®, Zoetis, Sao Paulo, Brazil) was inserted in them that remained in the animal until day -2.5. On the same day (-2.5), the recipients received i.m. 150 mg of D-cloprostenol (Croniben®, Biogenesis-Bagó, Curitiba, Brazil) and 1 mg of estradiol cypionate (ECP®; Zoetis, Sao Paulo, Brazil) and were then randomly divided into two groups (Fig. 1): **the eCG-group** ($n = 179$) in which cows were treated i.m. with 300 IU of equine chorionic gonadotropin (Novormon®; Zoetis, Sao Paulo, Brazil) and the **hCG-group** ($n = 162$) in which females cows i.m. 150 IU of human chorionic gonadotropin (Vetecor®, Ceva, Paulinia, Brazil).

Fig. 1 - Experimental design and hormonal protocol for timed embryo transfer (TET) program. *Bos indicus-taurus* recipients were synchronized by TET protocol and received gonadotropic support with equine chorionic gonadotropin (eCG-group) or human chorionic gonadotropin (hCG-group). (EB – estradiol benzoate; P4 – progesterone; PGF2α – D-cloprostenol; EC – estradiol cypionate; eCG – equine chorionic gonadotrophin; hCG – human chorionic gonadotropin; DF – dominant follicle; CL – corpus luteum).



2.3. Manifestation of estrus and ultrasound evaluations

On day 0, 261 recipients (eCG = 137 and hCG = 124) were evaluated to identify the manifestation of estrus, and 152 females were evaluated for the presence and diameter of the dominant follicle using ultrasound examination (SonoScape ™, Model S8, Domed, Valinhos, Brazil). To assess the manifestation of estrus, after removal of the P4 device (Day -2.5), the sacrococcygeal region was painted following the method described by Nogueira et al. [45]. On day 0, the recipients were classified as high estrus (paint completely removed), low estrus (paint partially removed), and no estrus (paint was not removed). A dominant follicle (DF) was defined as a follicle ≥ 8 mm in diameter and that exceeded the diameter of other follicles. The diameter was measured using ultrasonography and calculated from two cross-sectional linear measurements of the follicular antrum captured on the ultrasound monitor.

On Day 7, each female was evaluated by ultrasound, and those with at least one CL were considered acceptable in the TEF program and received an embryo ipsilateral to the CL. The embryos were produced in a commercial laboratory by *in vitro* embryo production (IVEP), being inovulated to fresh and at the blastocyst stage (day 7).

Before embryo transfer, 225 recipients considered acceptable were evaluated using B-mode ultrasound to measure the diameter (mean of two cross-sectional linear), area, and perimeter of the

CL. The area and luteal perfusion of the CL were evaluated using color Doppler ultrasound (SonoScape™, Model S8, Domed, Valinhos, Brazil) equipped with a 5-10-MHz transrectal linear transducer. A single operator performed the assessments and the device was configured to detect a blood flow of 5.4 cm/s.

The diameter, area, and perimeter of the CL were measured using a static image in B-mode and the largest diameter was measured through the ellipse function of the ultrasound. To determine the flow scores, the presence of each colored dot in the luteal structure was considered and the percentage of blood flow to each CL was estimated in relation to the total area of the CL. For cavity CLs, the CL area was determined from the total area minus the area of the luteal cavity.

For Doppler evaluation, a single trained operator performed all scans, and the CL of each female was subjectively evaluated based on the central and peripheral flow scores and the final Doppler scores. To assess the central flow, a score classification (0 to 4) was performed, as proposed by Pugliesi et al. [46]. Briefly, a score of 0 to 1 (scarce or non-existent central flow) represents less than 10% of the central area of the CL, a score of 2 represents central flow in up to 25% of the central area of the CL, a score of 3 represents central flow in 35% or greater but less than 45% of the central area of the CL, and a score of 4 represents central flow in greater than 50% of the central area of the CL.

The peripheral flow was evaluated using a score classification (0 to 4) similar to the central flow. A score of 0 to 1 (scarce or non-existent peripheral flow) represents peripheral flow in less than 10% of the peripheral area of the CL, a score of 2 represents peripheral flow in up to 25% of the peripheral area of the CL, a score of 3 represents peripheral flow in 35% or greater but less than 45% of the peripheral area of the CL, and a score of 4 represents peripheral flow in greater than 50% of the peripheral area of the CL.

Thus, based on the central and peripheral flow scores, the CL of each recipient was ranked from 1 to 3, as proposed by Pugliesi et al. [47]. Score 1: the presence of vascularization in up to 40% of the luteal area, score 2: the presence of vascularization in greater than 45% and less than 50% of the luteal area; and score 3: the presence of vascularization in greater than 55% of the luteal area.

Pregnancy diagnosis was performed using transrectal ultrasound on day 30, that is, on the 23rd day after embryo transfer. All the transfers were performed by one technician.

2.4. Statistical analysis

The BCS, diameter of the dominant follicle, other size variables (diameter, perimeter, and area), and CL quality (central and peripheral flow and CL Doppler score) were evaluated by ANOVA using a mixed-effect model. To compose the models in each analysis, gonadotropic treatment (eCG *vs.* hCG-main effect), parity order (nulliparous *vs.* multiparous cows), and intensity of estrus expression (high *vs.* low/absent) were considered fixed effects. Animals were considered random effects, and other sources of variation were included as covariates of the model. In the presence of significant effects, Tukey's test was used as the post-hoc mean test.

The rates of estrus expression, utilization (recipients with CL), conception, and pregnancy were analyzed using a binary logistic regression model including gonadotropic treatment and parity order as fixed effects. BCS was considered a covariate of the model. In the presence of a significant effect, ranking between groups was established using a 2 × 2 ratio test. In both models, all the main effects and possible interactions were considered. For descriptive analysis, quantitative data were presented as mean (M) and standard error (SE), and qualitative data as percentages (%). All statistical analyses were performed using Minitab® statistical program version 18.1. The level of significance was set at 5%; therefore, a significance level ≤ 0.05 indicated an effect of categorical variables and their interactions, and the statistical tendency was considered when P < 0.10 > 0.06.

3. Results

3.1. Diameter of the dominant follicle and rate of estrus expression

The BCS was similar (P = 0.43) between cows from the eCG (3.46 ± 0.03) and hCG group (3.44 ± 0.03) and did not affect the results of this study. The diameter of the dominant follicle on day 0 of the TET protocol was not influenced by gonadotropic treatment (P = 0.18; Table 1). However, an effect of parity order (multiparous or nulliparous) (P = 0.04) and the interaction between gonadotropic treatment and parity (P = 0.01) was observed, wherein heifers treated with hCG had dominant follicle with the smallest diameter on day 0 (Table 3).

Table 1 - Diameter of the dominant follicle (DF) in embryo recipients from different categories treated with equine chorionic gonadotropin (eCG) or equine chorionic gonadotropin (hCG) during the timed embryo transfer protocol

Variables		n	Diameter of the DF on Day 0 (mm) (M ± EP)
Treatment	eCG	89	10.28 ± 0.20
	hCG	63	9.83 ± 0.26
	Total/P-value	152	0.18
Parity	Multiparous	72	10.39 ± 0.23

	Nulliparous	80	9.67 ± 0.22
	Total/P-value	152	0.04
Interaction	Treatment*parity		0.01

In both the eCG and hCG groups, a greater proportion of females ($P < 0.05$) showed a high intensity of estrus expression than low/absent estrus intensity (Fig. 2). However, the proportion of females having a high intensity of estrus expression was higher in the recipients treated with hCG than in those treated with eCG ($P = 0.03$). The category ($P = 0.27$) did not influence estrus expression.

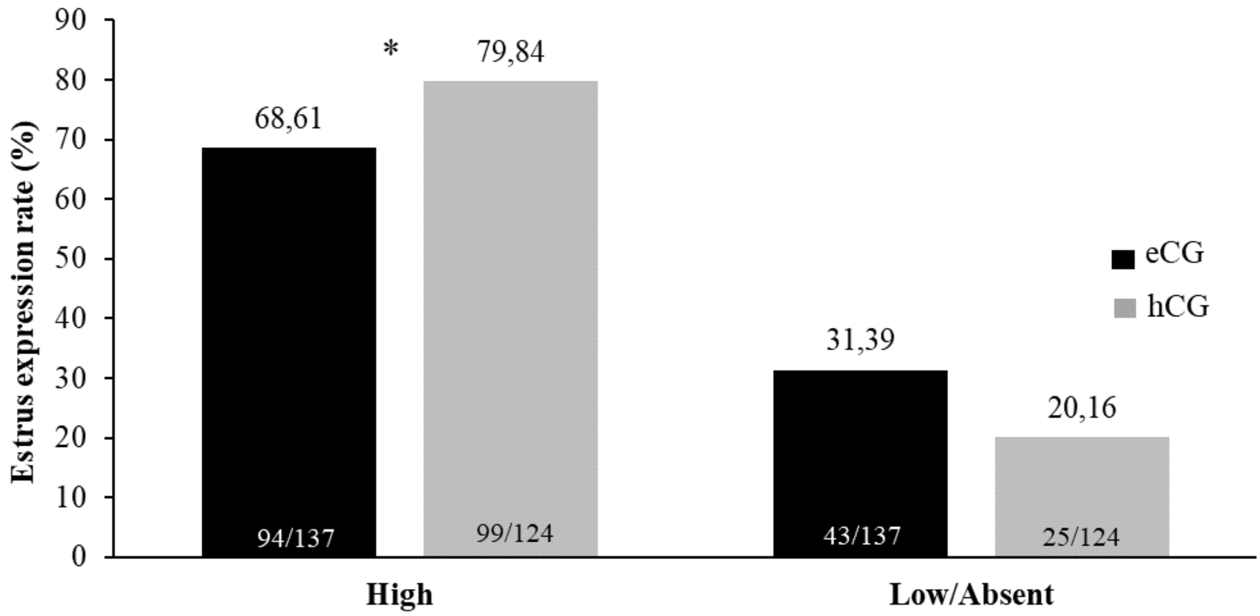


Fig. 2 - Intensity of estrus expression in embryo recipients treated with equine chorionic gonadotropin (eCG) or human chorionic gonadotropin (hCG) during the timed embryo transfer protocol.
* indicates a difference ($P = 0.03$) between the eCG and hCG groups classified as having a high intensity of estrus expression.

3.2. Utilization rate in recipients

The utilization rate (recipients with CL) showed a tendency to gonadotropic treatment ($P = 0.07$) and an effect to the parity order ($P = 0.001$). Moreover, a tendency of the interaction between gonadotropic treatment and parity ($P = 0.06$) was observed, wherein heifers treated with hCG had a lower utilization rate than those treated with eCG (Table 2).

Table 2 - Utilization rate of embryo recipients from different categories (nulliparous and multiparous) treated with equine chorionic gonadotropin (eCG) or human chorionic gonadotropin (hCG) during the timed embryo transfer protocol.

Variables		**n**	**Utilization rate % (n)**
Treatment	eCG	179	84.36 (151)
	hCG	162	77.16 (125)
	Total/P-value	341	0.07
Parity	Multiparous	147	85.71 (126)
	Nulliparous	194	77.32 (150)
	Total/P-value	341	0.001
Interaction	Treatment*parity		0.06

Table 3 - Effect of the interaction between gonadotropic treatment [equine chorionic gonadotrophin (eCG) or human chorionic gonadotrophin (hCG)] and parity order (multiparous or nulliparous) on the diameter of the dominant follicle (DF) on Day 0, utilization rate, conception rate and pregnancy rate of recipients submitted to timed embryo transfer protocol.

Interaction groups	**Diameter of the DF on Day 0 (mm) M ± EP**	**Utilization rate % (n/N)**	**Conception rate % (n/N)**	**Pregnancy rate % (n/N)**
eCG*multiparous	10.33 ± 0.32 [a]	88.89 [a] (64/72)	40.30 [ab] (27/67)	37.50 [a] (27/72)
eCG*nulliparous	10.44 ± 0.26 [a]	81.31 [ab] (87/107)	29.76 [bc] (25/84)	24.30 [b] (26/107)
hCG*multiparous	10.73 ± 0.35 [a]	82.67 [ab] (62/75)	44.26 [a] (27/61)	37.33 [a] (28/75)
hCG*nulliparous	9.33 ± 0.36 [b]	72.41 [b] (63/87)	18.03 [c] (11/61)	13.79 [c] (12/87)
P-value	0.01	0.06	0.01	0.001

a-c indicates statistical difference ($P < 0.05$) among interaction groups.

The diameter, perimeter, and area of the CL were similar (P > 0.1) between recipients treated with eCG or hCG (Table 4). None of these variables were influenced by parity order (P > 0.1). However, recipients who received hCG had CL with a better Doppler evaluation score (P = 0.04), central blood flow (P = 0.03), and a tendency towards greater peripheral blood flow (P = 0.08). Parity order did not influence (P > 0.1) the CL Doppler variables.

Table 4 - Evaluation of the corpus luteum (CL), assessed by B-mode and Doppler ultrasound, in embryo recipients treated with equine chorionic gonadotropin (eCG) or equine chorionic gonadotropin (hCG) during the timed embryo transfer protocol.

Variables	**eCG** M ± EP	**hCG** M ± EP	**P-value**
Animals (n)	123	102	-
Diameter (mm)	17.56 ± 0.31	16.98 ± 0.39	0.20
Perimeter (mm)	55.44 ± 1.02	53.52 ± 1.22	0.19
Area (mm^2)	247.80 ± 8.09	230.20 ± 11.00	0.17
Central flow	1.65 ± 0.07	1.90 ± 0.08	0.03
Peripheral flow	1.97 ± 0.07	2.16 ± 0.08	0.08
CL Doppler score	1.62 ± 0.07	1.83 ± 0.08	0.04

The conception rate in recipients treated with eCG was similar (P = 0.46; Fig. 3) to that in those treated with hCG; however, an effect of parity order (P = 0.01) and CL Doppler score (P = 0.02) was observed. Furthermore, an interaction was also found between gonadotropic treatment and animal parity (P = 0.003; Table 3) and gonadotropic treatment and CL Doppler score (P = 0.05; Fig. 4).

The pregnancy rate of recipients treated with eCG was similar (P = 0.20; Fig. 3) to that of those treated with hCG; however, an effect of parity order [nulliparous 25.0% (38/152) *vs.* multiparous 41.7% (55/132); P = 0.001] was observed. An interaction between gonadotropic treatment and parity was also observed (P = 0.001; Table 3).

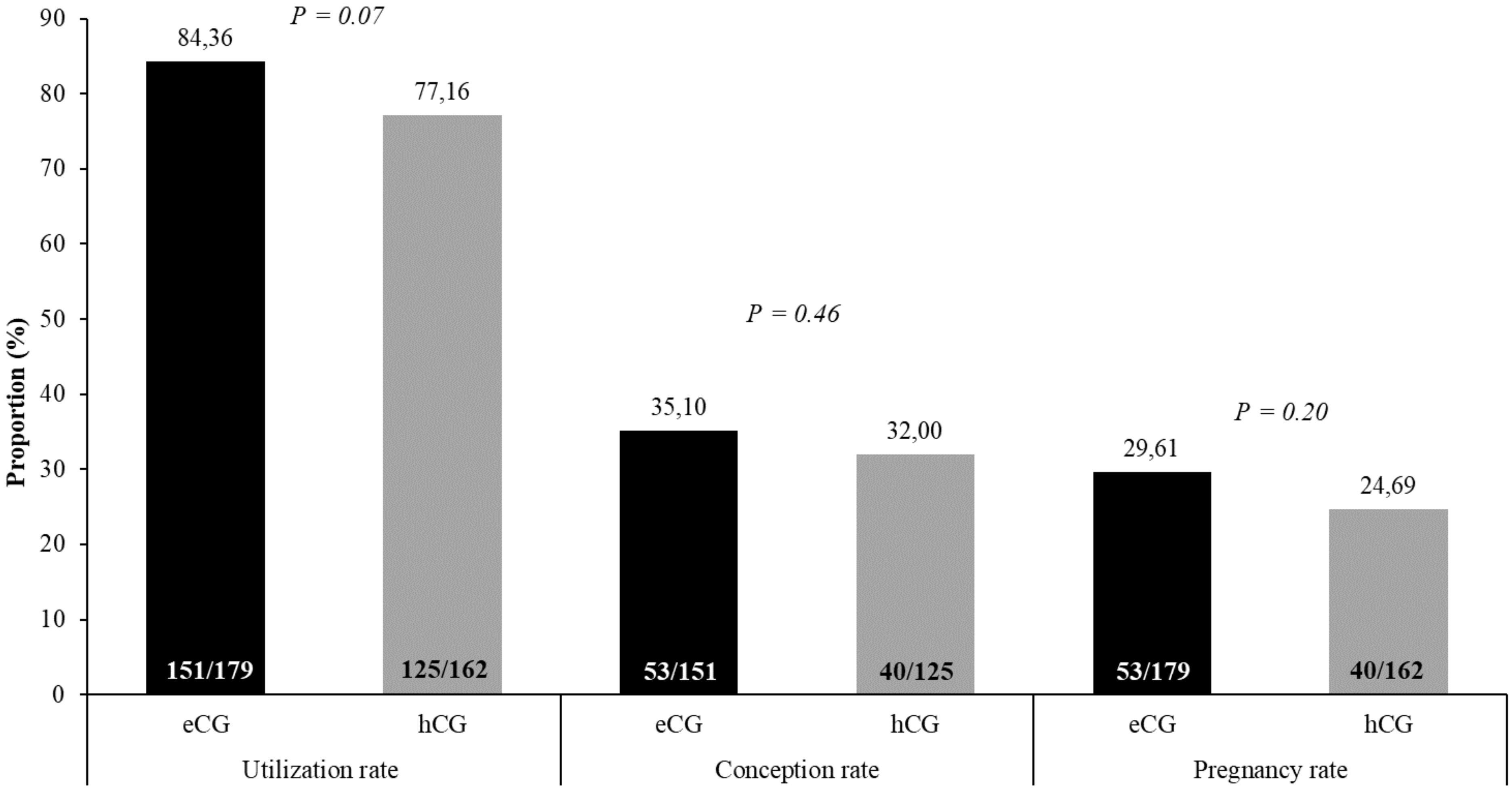


Fig. 3 - Utilization, conception, and pregnancy rates of embryo recipients submitted to timed embryo transfer protocol using equine chorionic gonadotropin (eCG) or human chorionic gonadotropin (hCG).

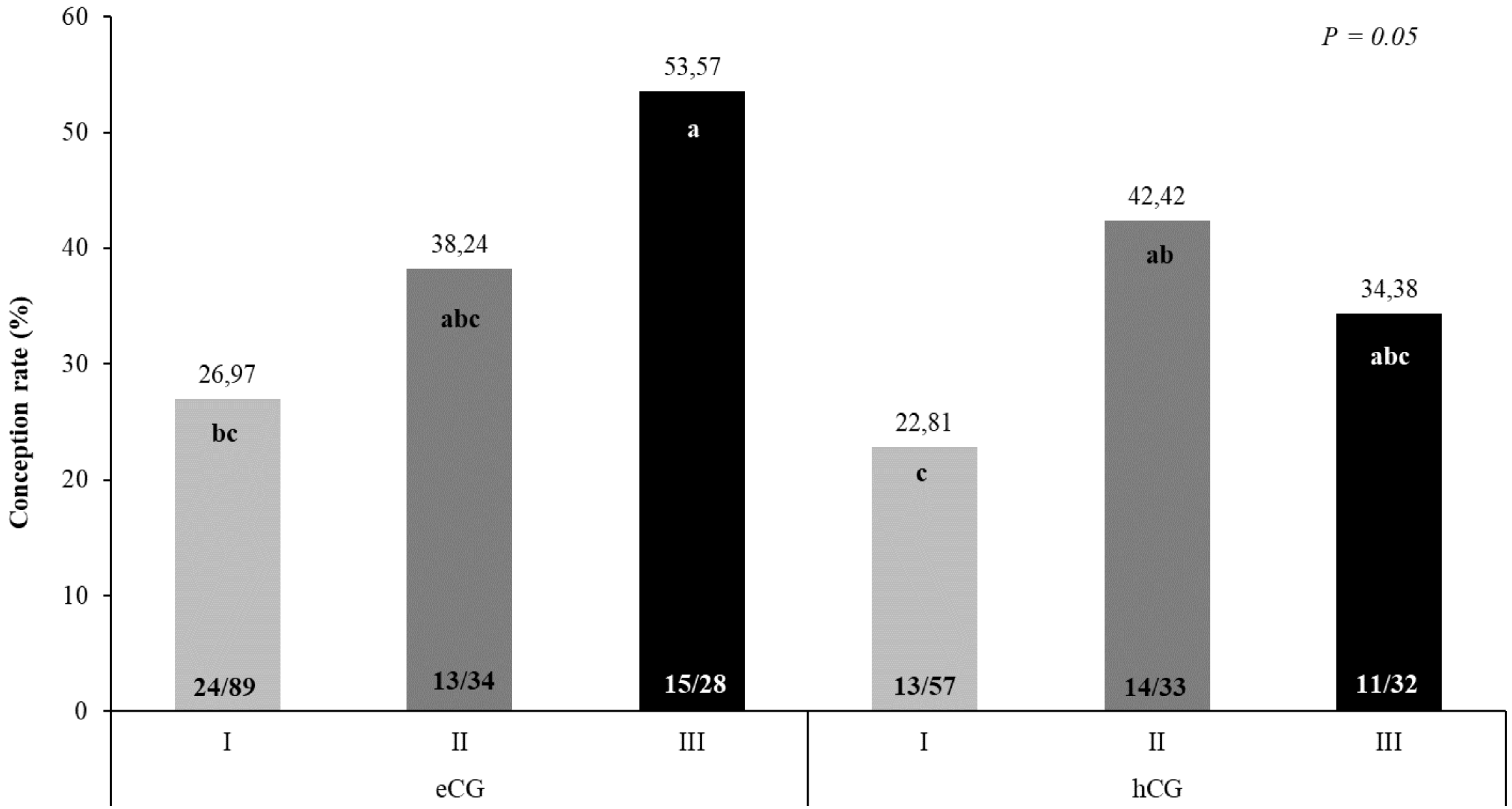


Fig. 4 - Effect of the interaction between gonadotropic treatment [equine chorionic gonadotrophin (eCG) or human chorionic gonadotrophin (hCG)] and corpus luteum Doppler evaluation score (I, II, or III) on conception rate in recipients submitted to timed embryo transfer protocol.

a-c indicates statistical difference ($P < 0.05$) among groups.

4. Discussion

To the best of our knowledge, this is the first study to evaluate the use of hCG as a replacement for eCG during the ovulation synchronization protocol for TET programs. We demonstrated that in addition to representing a little-explored strategy in TEF programs, gonadotropic stimulation with hCG proved to be a promising strategy at least in multiparous recipients as it resulted in the same diameter of the dominant follicle, utilization rate, corpus luteum quality, and conception and pregnancy rates as those obtained with eCG. However, eCG was more efficient than hCG in nulliparous embryo recipients during the TET program.

The dominant follicle with the smallest diameter, 48h after the TET protocol (day 0 in this study), was found in nulliparous heifers treated with hCG. This may be because gonadotropic stimulation with hCG was not as effective as eCG in promoting follicular growth in nulliparous heifers. Therefore, the effect of hCG treatment is comparable to that of eCG treatment in multiparous but not nulliparous cows. The large diameter of the dominant follicle after the ovulation synchronization protocol is positively related to several aspects related to female fertility [48-50] as well as greater estrus expression [45, 48], higher ovulatory rate [14, 48], larger CL size, and higher P4 concentration [51], in addition to a higher conception rate after insemination [48, 49, 52, 53].

Both eCG and hCG treatments resulted in a high proportion of recipients with a high intensity of estrus expression. However, compared to eCG, hCG contributed to a higher proportion of females with high intensity of estrus expression. This may be related to the high binding capacity of hCG to LH receptors, as suggested by Yavas et al. [54] and Binversie et al. [55], contributing to high steroidogenesis [26, 42, 56]; however, this phenomenon needs to be further investigated.

Both eCG and hCG exhibit dual gonadotropic activities (binding to FSH and LH receptors). However, eCG has a longer half-life and higher affinity for FSH [56], whereas hCG has a shorter half-life and higher affinity for LH [57]. Greater LH action with hCG treatment may have produced greater steroidogenic activity, contributing to increased intensity of estrus expression, in addition to contributing to the formation of a CL with a higher blood flow Doppler score, as shown in the present study, as well as by De Rensis and López-Gatius [26] and Lamb et al. [10]. However, it appears that FSH stimulation is more important in nulliparous females. Perhaps, in this category, it is necessary to adjust the dose or time of hCG treatment.

In the present study, a utilization rate of approximately 72 to 89% was similar to that observed by Bó et al. [58], who found a utilization rate of 75% to 85% using a conventional TET protocol based on P4-E2 and eCG. The interaction between gonadotropin treatment and parity order also played a role, wherein nulliparous recipients with hCG showed the lowest utilization rate. This may be related to the physiological aspects that limit the reproductive performance of females who are not fully sexually mature. In young females, 17β-estradiol exerts negative feedback on the hypothalamus and inhibits GnRH secretion [59, 60]. With sexual maturity, there is a decrease in the number of cytoplasmic 17β-estradiol receptors present in the hypothalamus and adenohypophysis, reducing the negative feedback mechanism and increasing GnRH release. This increases LH pulsatility, causing the final growth and maturation of the dominant follicle and induction of ovulation [59, 61].

It is expected that heifers will gradually adjust the mechanisms involved in the functioning of the hypothalamic-pituitary-gonadal axis. Such changes affect hormonal bioavailability and responsiveness of reproductive organs to pharmacological protocols. This can be explored by analyzing the effects of eCG treatment in nulliparous recipients that lead to a large follicular diameter and utilization rate because increased FSH activity due to eCG is beneficial for follicular growth. In contrast, with hCG, the growing follicles may have not been as responsive to the increased LH activity due to hCG, which is why their development was suppressed and ovulation was prevented. One possibility to be investigated for the optimization of the TET protocol with hCG in heifers is to pre-synchronize these animals to challenge them to improve their performance. Nevertheless, we established that eCG is the most recommended strategy in TET programs for nulliparous recipients.

The size of the CL in the recipients in the present study was similar regardless of the gonadotropin treatment or parity order. However, the CL Doppler evaluation score and central and peripheral blood flow were higher in recipients that underwent hCG treatment. This result is positive because these variables assess the functionality of reproductive organs and tissues by characterizing blood flow [46, 47, 62]. Larger areas of vascularization of the CL are associated with higher serum concentrations of P4 [63-67], which have been associated with lower rates of early pregnancy loss and better reproductive performance [68-70]. Although the present study did not assess P4 dosage, the conception rate observed supports the hypothesis that CLs with higher Doppler evaluation result in better conception rates regardless of gonadotropic treatment (Fig. 4;

CL score I ≈ 22-27% *versus* CL score II and III ≈ 34-54%). Corroborating this study, a previous study showed that recipients with high vascularization of the CL (> 40%) achieved a pregnancy rate of 48.4%, whereas those with low vascularization (< 40%) did not become pregnant [71]. Pugliesi et al. [62] also found an increase in pregnancy rates in recipients with a high degree of vascularization of the CL (low, 45.1%; medium, 55.9%; high, 62.3%).

Overall, conception (eCG, 35%; hCG, 32%) and pregnancy (eCG, 29%; hCG, 24%) rates were similar for the two gonadotropins evaluated, which may be associated with the stimulation of follicular growth and greater luteotrophic effect of these agents [18, 24, 29]. An interaction between gonadotropic treatment and Doppler CL assessment score was observed to the conception rate, suggesting that the TET protocol with eCG or hCG seems to be more effective to increase the conception in recipients with better CL Doppler scores.

5. Conclusion

In conclusion, hCG is a viable option for gonadotropic stimulation in TET programs with multiparous recipients as it resulted in a greater intensity of estrus expression and CL with a higher Doppler score, which promoted utilization, conception, and pregnancy at rates comparable to conventional protocols that use eCG. However, we highlighted that hCG results in lower overall reproductive rates than eCG in nulliparous category, and thus, its use is recommended for multiparous recipients alone.

CRediT authorship contribution statement

E.A.A. Rossignolo: Conceptualization, Methodology, First Draft; N.C. Silva: Data Curation, Organization, Methodology and First Draft; R.L. Stolf: Data Curation and Collection; F.L.B. Cavalieri: Conceptualization, Methodology, Interpretation, Funding Acquisition and Resources; A.H.B. Colombo: Data Curation and Methodology; M.A. Andreazzi: Data Curation and Methodology; M.M. Seneda: Validation, Funding Acquisition, Resources, Writing - Review & Editing; F. Morotti: Conceptualization, Formal Analysis, Project Administration, Supervision, Writing - Review & Editing.

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Competing interests

The authors declare no competing interests.

References

[1] Mapletoft RJ, Bó GA, Baruselli PS, Menchaca A, Sartori R. Evolution of knowledge on ovarian physiology and its contribution to the widespread application of reproductive biotechnologies in South American cattle. Anim Reprod 2018;15:1003-14.

[2] Baruselli P, Sales J, Sala R, Vieira L, Sá Filho M. History, evolution and perspectives of timed artificial insemination programs in Brazil. Anim Reprod 2018;9:139-52.

[3] Baruselli PS, Ferreira RM, Colli MHA, Elliff FM, Sá Filho MF, Vieira L, et al. Timed artificial insemination: current challenges and recent advances in reproductive efficiency in beef and dairy herds in Brazil. Anim Reprod 2018;14:558-71.

[4] Stevenson J, Britt J. A 100-Year Review: Practical female reproductive management. J Dairy Sci 2017;100:10292-313.

[5] Vasconcelos JLM, Pereira MHC, Wiltbank MC, Guida TG, Lopes Jr FR, Sanches Jr CP, et al. Evolution of fixed-time AI in dairy cattle in Brazil. Anim Reprod 2018;15:940-51.

[6] Sales JN, Carvalho JB, Crepaldi GA, Cipriano RS, Jacomini JO, Maio JR, et al. Effects of two estradiol esters (benzoate and cypionate) on the induction of synchronized ovulations in Bos indicus cows submitted to a timed artificial insemination protocol. Theriogenology 2012;78:510-6.

[7] Siqueira LC, Oliveira JF, Rovani MT, Ferreira R, Borges LF, Gonçalves PB. Effects of estradiol and progestins on follicular regression before, during, and after follicular deviation in postpartum beef cows. Theriogenology 2009;71:614-9.

[8] Madureira G, Consentini CE, Motta JC, Drum JN, Prata AB, Monteiro Jr PL, et al. Progesterone-based timed AI protocols for Bos indicus cattle II: reproductive outcomes of either EB or GnRH-type protocol, using or not GnRH at AI. Theriogenology 2020;145:86-93.

[9] Rodrigues WB, Silva AS, Silva JCB, Anache NA, Silva KC, Cardoso CJT, et al. Timed artificial insemination plus heat II: gonadorelin injection in cows with low estrus expression scores increased pregnancy in progesterone/estradiol-based protocol. Animal 2019;13:2313-8.

[10] Lamb GC, Dahlen CR, Larson JE, Marquezini G, Stevenson JS. Control of the estrous cycle to improve fertility for fixed-time artificial insemination in beef cattle: a review. J Anim Sci 2010;88:E181-92.

[11] Bó GA, Baruselli PS. Synchronization of ovulation and fixed-time artificial insemination in beef cattle. Animal 2014;8 Suppl 1:144-50.

[12] Madureira G, Motta JC, Drum JN, Consentini CE, Prata AB, Monteiro Jr PL, et al. Progesterone-based timed AI protocols for Bos indicus cattle I: evaluation of ovarian function. Theriogenology 2020;145:126-37.

[13] Marques MO, Morotti F, da Silva CB, Júnior MR, da Silva RC, Baruselli PS, et al. Influence of category--heifers, primiparous and multiparous lactating cows--in a large-scale resynchronization fixed-time artificial insemination program. J Vet Sci 2015;16:367-71.

[14] Gimenes LU, MF SF, Carvalho NA, Torres-Júnior JR, Souza AH, Madureira EH, et al. Follicle deviation and ovulatory capacity in Bos indicus heifers. Theriogenology 2008;69:852-8.

[15] Sá Filho MF, Baldrighi JM, Sales JN, Crepaldi GA, Carvalho JB, Bó GA, et al. Induction of ovarian follicular wave emergence and ovulation in progestin-based timed artificial insemination protocols for Bos indicus cattle. Anim Reprod Sci 2011;129:132-9.

[16] Nasser L, Reis E, Oliveira M, Bó G, Baruselli P. Comparison of four synchronization protocols for fixed-time bovine embryo transfer in Bos indicus× Bos taurus recipients. Theriogenology 2004;62:1577-84.

[17] Baruselli PS, Ferreira RM, Sales JN, Gimenes LU, MF SF, Martins CM, et al. Timed embryo transfer programs for management of donor and recipient cattle. Theriogenology 2011;76:1583-93.

[18] Bó GA, Peres LC, Cutaia LE, Pincinato D, Baruselli PS, Mapletoft RJ. Treatments for the synchronisation of bovine recipients for fixed-time embryo transfer and improvement of pregnancy rates. Reprod Fertil Dev 2011;24:272-7.

[19] Bó GA, Cedeño A. Expression of estrus as a relevant factor in fixed-time embryo transfer programs using estradiol/progesterone-based protocols in cattle. Anim Reprod 2018;15:224-30.

[20] Yavas Y, Walton JS. Postpartum acyclicity in suckled beef cows: a review. Theriogenology 2000;54:25-55.

[21] Montiel F, Ahuja C. Body condition and suckling as factors influencing the duration of postpartum anestrus in cattle: a review. Anim Reprod Sci 2005;85:1-26.

[22] Campos JT, Marinho LS, Lunardelli PA, Morotti F, Seneda MM. Resynchronization of estrous cycle with eCG and temporary calf removal in lactating Bos indicus cows. Theriogenology 2013;80:619-23.

[23] Barreiros TR, Blaschi W, Santos GM, Morotti F, Andrade ER, Baruselli PS, et al. Dynamics of follicular growth and progesterone concentrations in cyclic and anestrous suckling Nelore cows (Bos indicus) treated with progesterone, equine chorionic gonadotropin, or temporary calf removal. Theriogenology 2014;81:651-6.

[24] Baruselli PS, Reis EL, Marques MO, Nasser LF, Bó GA. The use of hormonal treatments to improve reproductive performance of anestrous beef cattle in tropical climates. Anim Reprod Sci 2004;82-83:479-86.

[25] Murphy BD, Martinuk SD. Equine chorionic gonadotropin. Endocr Rev 1991;12:27-44.

[26] De Rensis F, López-Gatius F. Use of equine chorionic gonadotropin to control reproduction of the dairy cow: a review. Reprod Domest Anim 2014;49:177-82.

[27] Thatcher WW, Moreira F, Santos JE, Mattos RC, Lopes FL, Pancarci SM, et al. Effects of hormonal treatments on reproductive performance and embryo production. Theriogenology 2001;55:75-89.

[28] Takahashi T, Hirako M, Patel OV, Shimizu M, Hasegawa Y, Domeki I, et al. Plasma steroid profiles following follicle-stimulating hormone or equine chorionic gonadotropin injection in cows chronically treated with gonadotropin-releasing hormone agonist. J Vet Med Sci 2002;64:731-4.

[29] Baruselli P, Ferreira R, Sales J, Gimenes L, Sá Filho M, Martins C, et al. Timed embryo transfer programs for management of donor and recipient cattle. Theriogenology 2011;76:1583-93.

[30] Hoppen HO. The equine placenta and equine chorionic gonadotrophin: an overview. Exp Clin Endocrinol 1994;102:235-43.

[31] Villarraza CJ, Antuña S, Tardivo MB, Rodríguez MC, Mussio P, Cattaneo L, et al. Development of a suitable manufacturing process for production of a bioactive recombinant equine chorionic gonadotropin (reCG) in CHO-K1 cells. Theriogenology 2021;172:8-19.

[32] Manteca Vilanova X, De Briyne N, Beaver B, Turner PV. Horse Welfare During Equine Chorionic Gonadotropin (eCG) Production. Animals : an open access journal from MDPI 2019;9:1053.

[33] De Rensis F, López-Gatius F, García-Ispierto I, Techakumpu M. Clinical use of human chorionic gonadotropin in dairy cows: An update. Theriogenology 2010;73:1001-8.

[34] de Medeiros SF, Amato F, Matthews CD, Norman RJ. Urinary concentrations of beta core fragment of hCG throughout pregnancy. Obstet Gynecol 1992;80:223-8.

[35] Ireland J, Roche J. Development of antral follicles in cattle after prostaglandin-induced luteolysis: changes in serum hormones, steroids in follicular fluid, and gonadotropin receptors. Endocrinology 1982;111:2077-86.

[36] Ireland J, Roche J. Growth and differentiation of large antral follicles after spontaneous luteolysis in heifers: changes in concentration of hormones in follicular fluid and specific binding of gonadotropins to follicles. J Anim Sci 1983;57:157-67.

[37] Choi J, Smitz J. Luteinizing hormone and human chorionic gonadotropin: Origins of difference. Mol Cell Endocrinol 2014;383:203-13.

[38] Burns MG, Buttrey BS, Dobbins CA, Martel CA, Olson KC, Lamb GC, et al. Evaluation of human chorionic gonadotropin as a replacement for gonadotropin-releasing hormone in ovulation-synchronization protocols before fixed timed artificial insemination in beef cattle. J Anim Sci 2008;86:2539-48.

[39] Giordano JO, Wiltbank MC, Guenther JN, Ares MS, Lopes G, Jr., Herlihy MM, et al. Effect of presynchronization with human chorionic gonadotropin or gonadotropin-releasing hormone 7 days before resynchronization of ovulation on fertility in lactating dairy cows. J Dairy Sci 2012;95:5612-25.

[40] Nascimento AB, Bender RW, Souza AH, Ayres H, Araujo RR, Guenther JN, et al. Effect of treatment with human chorionic gonadotropin on day 5 after timed artificial insemination on fertility of lactating dairy cows. J Dairy Sci 2013;96:2873-82.

[41] Besbaci M, Abdelli A, Minviel JJ, Belabdi I, Kaidi R, Raboisson D. Association of pregnancy per artificial insemination with gonadotropin-releasing hormone and

human chorionic gonadotropin administered during the luteal phase after artificial insemination in dairy cows: A meta-analysis. J Dairy Sci 2020;103:2006-18.

[42] Cabrera EM, Lauber MR, Peralta EM, Bilby TR, Fricke PM. Human chorionic gonadotropin dose response for induction of ovulation 7 days after a synchronized ovulation in lactating Holstein cows. JDS Communications 2021;2:35-40.

[43] Prata AB, Drum JN, Melo LF, Araujo ER, Sartori R. Effect of different chorionic gonadotropins on final growth of the dominant follicle in Bos indicus cows. Theriogenology 2018;111:52-5.

[44] Souza A, Valenza A, Carneiro T, Zanatta G, Mingoti R, Silva L, et al. A low dose of hCG (150IU) associated to inducing ovulation with estradiol benzoate can successfully replace eCG in timed AI protocols in Nelore cows Animal Reprodution. Ilha de Comandatuba, BA, Brazil, 2019. p. 559.

[45] Nogueira E, Silva MR, Silva JCB, Abreu UPG, Anache NA, Silva KC, et al. Timed artificial insemination plus heat I: effect of estrus expression scores on pregnancy of cows subjected to progesterone-estradiol-based protocols. Animal 2019;13:2305-12.

[46] Pugliesi G, Rezende RG, Silva Jd, Lopes E, Nishimura TK, Baruselli PS, et al. Uso da ultrassonografia Doppler em programas de IATF e TETF em bovinos. Rev Bras Reprod Anim 2017;41:140-50.

[47] Pugliesi G, de Melo GD, Silva JB, Carvalhêdo AS, Lopes E, de Siqueira Filho E, et al. Use of color-Doppler ultrasonography for selection of recipients in timed-embryo transfer programs in beef cattle. Theriogenology 2019;135:73-9.

[48] Sa Filho MF, Crespilho AM, Santos JE, Perry GA, Baruselli PS. Ovarian follicle diameter at timed insemination and estrous response influence likelihood of ovulation and pregnancy after estrous synchronization with progesterone or progestin-based protocols in suckled Bos indicus cows. Anim Reprod Sci 2010;120:23-30.

[49] Meneghetti M, OG SF, Peres RF, Lamb GC, Vasconcelos JL. Fixed-time artificial insemination with estradiol and progesterone for Bos indicus cows I: basis for development of protocols. Theriogenology 2009;72:179-89.

[50] Perry GA, Smith MF, Lucy MC, Green JA, Parks TE, MacNeil MD, et al. Relationship between follicle size at insemination and pregnancy success. Proceedings of the National Academy of Sciences of the United States of America 2005;102:5268-73.

[51] Pfeifer LF, Mapletoft RJ, Kastelic JP, Small JA, Adams GP, Dionello NJ, et al. Effects of low versus physiologic plasma progesterone concentrations on ovarian follicular development and fertility in beef cattle. Theriogenology 2009;72:1237-50.

[52] Morotti F, Moretti R, Dos Santos GMG, Silva-Santos KC, Cerqueira PHR, Seneda MM. Ovarian follicular dynamics and conception rate in Bos indicus cows with different antral follicle counts subjected to timed artificial insemination. Anim Reprod Sci 2018;188:170-7.

[53] Pfeifer LFM, Castro NA, Melo VTO, Neves PMA, Cestaro JP, Schneider A. Timed artificial insemination in blocks: A new alternative to improve fertility in lactating beef cows. Anim Reprod Sci 2015;163:89-96.

[54] Yavas Y, Johnson WH, Walton JS. Modification of follicular dynamics by exogenous FSH and progesterone, and the induction of ovulation using hCG in postpartum beef cows. Theriogenology 1999;52:949-63.

[55] Binversie JA, Pfeiffer KE, Larson JE. Modifying the double-Ovsynch protocol to include human chorionic gonadotropin to synchronize ovulation in dairy cattle. Theriogenology 2012;78:2095-104.

[56] Murphy B. Equine chorionic gonadotropin: an enigmatic but essential tool. Anim Reprod 2018;9:223-30.

[57] Saleh M, Shahin M, Wuttke W, Gauly M, Holtz W. Pharmacokinetics of human chorionic gonadotropin after i.m. administration in goats (Capra hircus). Reproduction 2012;144:77-81.

[58] Bó GA, Cedeño A, Mapletoft RJ. Strategies to increment in vivo and in vitro embryo production and transfer in cattle. Anim Reprod 2019;16:411-22.

[59] Kinder JE, Bergfeld EG, Wehrman ME, Peters KE, Kojima FN. Endocrine basis for puberty in heifers and ewes. J Reprod Fertil Suppl 1995;49:393-407.

[60] Amstalden M, Alves BRC, Liu S, Cardoso RC, Williams GL. Neuroendocrine pathways mediating nutritional acceleration of puberty: insights from ruminant models. Frontiers in endocrinology 2011;2:109-.

[61] Atkins JA, Pohler KG, Smith MF. Physiology and endocrinology of puberty in heifers. Vet Clin North Am Food Anim Pract 2013;29:479-92.

[62] Pugliesi G, de Melo GD, Ataíde GA, Jr., Pellegrino CAG, Silva JB, Rocha CC, et al. Use of Doppler ultrasonography in embryo transfer programs: feasibility and field results. Anim Reprod 2018;15:239-46.

[63] Gómez-Seco C, Alegre B, Martínez-Pastor F, Prieto JG, González-Montaña JR, Alonso ME, et al. Evolution of the corpus luteum volume determined ultrasonographically and its relation to the plasma progesterone concentration after artificial insemination in pregnant and non-pregnant dairy cows. Vet Res Commun 2017;41:183-8.

[64] Vrisman DP, Bastos NM, Rossi GF, Rodrigues NN, Borges LPB, Taira AR, et al. Corpus luteum dynamics after ovulation induction with or without previous exposure to progesterone in prepubertal Nellore heifers. Theriogenology 2018;106:60-8.

[65] Siqueira LG, Arashiro EK, Ghetti AM, Souza ED, Feres LF, Pfeifer LF, et al. Vascular and morphological features of the corpus luteum 12 to 20 days after timed artificial insemination in dairy cattle. J Dairy Sci 2019;102:5612-22.

[66] Herzog K, Voss C, Kastelic JP, Beindorff N, Paul V, Niemann H, et al. Luteal blood flow increases during the first three weeks of pregnancy in lactating dairy cows. Theriogenology 2011;75:549-54.

[67] Siqueira LG, Areas VS, Ghetti AM, Fonseca JF, Palhao MP, Fernandes CA, et al. Color Doppler flow imaging for the early detection of nonpregnant cattle at 20 days after timed artificial insemination. J Dairy Sci 2013;96:6461-72.

[68] Ledoux D, Ponsart C, Grimard B, Gatien J, Deloche MC, Fritz S, et al. Sire effect on early and late embryonic death in French Holstein cattle. Animal 2014;9:766-74.

[69] Adriaens I, Saeys W, Huybrechts T, Lamberigts C, François L, Geerinckx K, et al. A novel system for on-farm fertility monitoring based on milk progesterone. J Dairy Sci 2018;101:8369-82.

[70] Mann GE, Lamming GE. Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanism in cows. Reproduction 2001;121:175-80.

[71] Pinaffi FL, Santos ÉS, Silva MGd, Maturana Filho M, Madureira EH, Silva LA. Follicle and corpus luteum size and vascularity as predictors of fertility at the time of artificial insemination and embryo transfer in beef cattle. Pesquisa Veterinária Brasileira 2015;35:470-6.

6. CONSIDERAÇÕES FINAIS

- A gonadotrofina coriônica humana mostrou-se uma opção á gonadotrofina coriônica equina, em multíparas. Embora para novilhas não se mostrou como opção para substituir o eCG.

- A ultrassonografia doppler colorido mostrou-se uma ferramenta útil em programas de transferência de embrião embora sua principal função foi apontada na qualificação de corpos lúteos mais adequados a receber o embrião.

- A hCG ainda necessita de mais estudos com nulíparas, principalmente na sinergia de dieta e fármaco para então mostrar-se uma opção viável a ser substituto do eCG.

7. ANEXOS