UNIVERSIDAD AUTÓNOMA AGRARIA ANTONIO NARRO

SUBDIRECCIÓN DE POSTGRADO

EFECTO DE DOS DOSIS DE hCG EN DIFERENTES TIEMPOS DESPUÉS DE LA IATF EN CABRAS ANOVULATORIAS Y EN TRANSICIÓN REPRODUCTIVA SOBRE LA LUTEOGÉNESIS E IMPLANTACIÓN EMBRIONARIA

Tesis

Que presenta JORGE ARTURO BUSTAMANTE ANDRADE

como requisito parcial para obtener el Grado de
DOCTOR EN CIENCIAS EN PRODUCCIÓN AGROPECUARIA

Torreón, Coahuila Septiembre 2022

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Septiembre 2022

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Elaborada por JORGE ARTURO BUSTAMANTE ANDRADE como requisito parcial para obtener el grado de Doctor en Ciencias en Producción Agropecuaria con la supervisión y aprobación del Comité de Asesoría

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EFECTO DE DOS DOSIS DE hCG EN DIFERENTES TIEMPOS DESPUÉS DE LA IATF EN CABRAS ANOVULATORIAS Y EN TRANSICIÓN REPRODUCTIVA SOBRE LA LUTEOGÉNESIS E IMPLANTACIÓN EMBRIONARIA

JORGE ARTURO BUSTAMANTE ANDRADE
Doctor en Ciencias en Producción Agropecuaria

UNIVERSIDAD AUTÓNOMA AGRARIA ANTONIO NARRO

Dr. Francisco Gerardo Véliz Deras
Director de tesis

Evaluamos el efecto de dos dosis de hCG (100 y 300 UI) en dos momentos (7 y 14 d) después de un protocolo de inseminación artificial a tiempo fijo (IATF) durante la temporada de anestro. (Abril, 25° N). Se utilizaron cabras multíparas-anovulatorias, con peso corporal (43.6 ± 5.7 kg) y condición corporal (1.86 ± 0.28 unidades) ubicadas en el norte de México (25° N, 103° O). Tras la confirmación de la inducción del celo, las cabras (n = 61) se sometieron a un procedimiento IATF. Después las cabras se distribuyeron aleatoriamente en cinco grupos: (1). G100-7 (n = 13) 100 UI, hCG 7 d post-FTAI, (2). G100-14 (n = 12) 100 UI hCG, 14 d post-IATF, (3). G300-7 (n = 12) 300 UI, hCG, 7 d post-FTAI, (4). G300-14 (n = 12) 300 UI hCG 14 d post-FTAI, y (5). CONT (n = 12) 0,5 mL de solución salina, 7 y 14 días después de IATF. Las variables tasa de fecundidad, índices de eficiencia embrionaria-1 y 2 se vieron favorecidas por el tratamiento G300-14, el área del cuerpo lúteo se vio favorecida ($p < 0.05$) tanto por G300-7 como por G300-14. En el segundo estudio el objetivo fue evaluar los mismos tratamientos, solo que en la etapa de transición reproductiva (Junio, 25° N). Las cabras (n=40) fueron sometidas a un protocolo de inducción al estro, posteriormente las cabras fueron inseminadas, luego las cabras fueron distribuidas aleatoriamente en cinco grupos experimentales: 1). G100-7 (n = 8) 100 UI hCG, 7 días post-IATF; 2). G100-14 (n = 8) 100 UI hCG, 14 días post-IATF; 3). G300-7 (n = 8) 300 UI hCG, 7 días post-IATF; 4). G300-14 (n = 8) 300 UI hCG, 14 días después de IATF; y 5). Cont (n = 8) 0,5 ml de solución salina, 7 y 14 días post-IATF. Las variables

área de cuerpo lúteo, tasa de implantación embrionaria, índice de eficiencia embrionaria 1 y 2, tasas de concepción, fertilidad, fecundidad, pérdidas fetales a los d 30, d 45 y total favorecieron G300-14. Tal estrategia reproductiva del uso de hCG durante la temporada de anestro profundo natural y en el período de transición reproductiva, es eficaz en la luteogénesis, reduce las pérdidas fetales tempranas y mejora la tasa y la eficiencia embrionaria, fundamentales en el éxito reproductivo de los sistemas de producción caprina marginales.

Palabras clave: cabras, reproducción, luteogenesis, implantación embrionaria, pérdidas fetales.

ABSTRACT

We evaluated the effect of two doses of hCG (100 and 300 IU) at two times (7 and 14 d) after a fixed-time artificial insemination (FTAI) protocol during the anoestrus season. (April, 25°N). Multiparous-anovulatory goats with body weight (43.6 ± 5.7 kg) and body condition (1.86 ± 0.28 units) located in northern Mexico (25° N, 103° W) were used. After confirmation of oestrus induction, the goats (n = 61) underwent an IATF procedure. The goats were then randomly distributed into five groups: (1). G100-7 (n = 13) 100 IU, hCG 7 d post-FTAI, (2). G100-14 (n = 12) 100 IU hCG, 14 days post-FTAI, (3). G300-7 (n = 12) 300 IU, hCG, 7 days post-FTAI, (4). G300-14 (n = 12) 300 IU hCG 14 d post-FTAI, and (5). CONT (n = 12) 0.5 mL of saline, 7 and 14 days after FTAI. The variables fertility rate, embryonic efficiency indices-1 and 2 were favored by the G300-14 treatment, the corpus luteum area was favored ($p < 0.05$) by both G300-7 and G300-14. In the second study, the objective was to evaluate the same treatments, only in the reproductive transition stage (June, 25° N). The goats (n=40) were subjected to an estrus induction protocol, subsequently the goats were inseminated, then the goats were randomly distributed into five experimental groups: 1). G100-7 (n = 8) 100 IU hCG, 7 days post-FTAI; two). G100-14 (n = 8) 100 IU hCG, 14 days post-FTAI; 3). G300-7 (n = 8) 300 IU hCG, 7 days post-FTAI; 4). G300-14 (n = 8) 300 IU hCG, 14 days after FTAI; and 5). Cont (n = 8) 0.5 ml of saline, 7 and 14 days post-FTAI. The variables corpus luteum area, embryo implantation rate, embryo efficiency index 1 and 2, conception rates, fertility, fecundity, fetal loss at d 30, d 45 and total favored G300-14. Such a reproductive strategy of the use of hCG during the season of natural deep anoestrus and in the reproductive transition period is effective in luteogenesis, reduces early fetal losses and improves the rate and embryonic efficiency, fundamental in the reproductive success of systems. of marginal goat production.

Keywords: goats, reproduction, luteogenesis, embryo implantation, fetal losses.

INTRODUCCIÓN

La estacionalidad en la reproducción es una adaptación evolutiva que muestran algunas especies de mamíferos, caracterizada por la presentación de actividad sexual durante un periodo específico del año para garantizar la sobrevivencia de su progenie, y por consecuencia los partos ocurren de manera estacional, cuando la disponibilidad en cantidad y calidad de la vegetación es mayor (Bronson, 1985; Bronson y Heideman, 1994; Malpaux, 2006).

Muestra de lo anterior la especie caprina se enfrenta a un periodo de anestro estacional que manifiestan la mayoría de los diferentes genotipos raciales; (Álvarez y Zarco, 2001), en este sentido las hembras caprinas adaptadas a regiones subtropicales como la Comarca Lagunera (26° LN) manifiestan un periodo de inactividad de marzo a mayo y una estación sexual que inicia en agosto y termina en febrero (Carrillo *et al.,* 2010; Contreras-Villarreal *et al.,* 2015; Alvarado-Espino *et al.,* 2016). Como consecuencia, la producción de leche, cabrito y derivados es estacional (Álvarez y Zarco, 2001; Holtz, 2005). Para contrarrestar dicho anestro, se han empleado hormonas exógenas como la gonadotropina coriónica humana (hCG) (Alvarado-Espino *et al;* 2016; Rodríguez-Martínez *et al.,* 2017; Alvarado-Espino *et al.,* 2019ab) para inducir la actividad reproductiva, además de que favorece el proceso de implantación embrionaria. La administración de hCG estimula directamente a los folículos ováricos en etapas avanzadas del desarrollo, desencadenando el proceso ovulatorio y la formación del CL en cabras en anestro (Kawate *et al.,* 2002; Alvarado-Espino *et al.,* 2016). La hCG se ha empleado también para incrementar los niveles plasmáticos de progesterona, las tasas de implantación embrionaria, la prolificidad y el crecimiento fetal (Catalano *et al.,* 2012).

La implantación embrionaria, mantenida por la progesterona secretada por el cuerpo lúteo, es un proceso fundamental en la gestación de las cabras (Vera *et al.,* 2013) por ello, cualquier disfunción lútea que genere bajos niveles de P4 durante las primeras etapas de la gestación, generará muertes embrionarias tempranas (Khan *et al.* 2009), lo cual afecta la fertilidad durante las primeras

semanas de gestación (Khan *et al.,* 2009; Fernandez *et al.,* 2019). A esta etapa se le conoce como “periodo crítico” y representa un problema en la productividad de los hatos caprinos, en mayor medida en aquellos manejados bajo condiciones extensivas. En la región de la Comarca Lagunera los hatos caprinos son manejados predominantemente bajo un sistema de producción de pastoreo semi-extensivo en la flora nativa con una duración entre 8-10 horas y encierro nocturno con agua y sales minerales a libre acceso (Escareño *et al.,* 2011).

HIPÓTESIS GENERALES

1. La administración de una dosis baja de hCG (100 UI) después de la IATF en cabras anovulatorias (abril) será efectiva en la eficiencia embrionaria y la luteogénesis.

2. La aplicación de una dosis alta de hCG (300 UI) después de la IATF en cabras anovulatorias en transición reproductiva (junio) será efectiva para disminuir las pérdidas fetales tempranas y mejoraría la eficiencia embrionaria.

OBJETIVOS GENERALES

1. Determinar el efecto de la administración de 100 y 300 UI de hCG en diferentes tiempos después de la IATF en cabras anovulatorias (abril) sobre la eficiencia embrionaria y la luteogénesis.

2. Evaluar el efecto de la administración de 100 y 300 UI de hCG en diferentes tiempos después de la IATF en cabras anovulatorias en transición reproductiva (junio) sobre las pérdidas fetales tempranas y en la eficiencia embrionaria.

REVISIÓN DE LITERATURA

La caprinocultura es una actividad productiva de suma importancia ya que ha permitido el desarrollo ganadero desde el inicio de la humanidad (Castel *et al.,* 2010) en diferentes ecosistemas; actualmente, a nivel mundial existe un inventario de cerca de 1,050 millones de cabezas, donde el continente americano concentra más de 38.0 millones de cabras, siendo Brasil y México los principales países con 10,6 y 8,8 millones de cabras respectivamente (FAOSTAT, 2020; SIAP 2020).

La mayor parte de los caprinos vive en condiciones climáticas extremas, presentando una mayor capacidad para convertir diferentes recursos alimenticios en leche y carne con un valor biológico superior al de otros rumiantes domésticos. (Navarrete-Molina *et al.,* 2020). En México, como en otras latitudes, la caprinocultura se considera una práctica pecuaria relacionada con productores de bajos recursos que obtienen un ingreso económico a partir de la venta de leche, carne y cabrito. Por ejemplo, la Comarca Lagunera, región agroecológica ubicada en el norte árido de México, destaca en la producción caprina a nivel nacional y posee un inventario de 392,407 cabezas (SIAP 2020), ocupando el primer lugar en producción de leche de cabra a nivel nacional (Escareño *et al.,* 2013; Isidro-Requejo *et al.,* 2019; Navarrete-Molina *et al.,* 2020), mostrando en los últimos años una reducción en el inventario y un incremento en la producción láctea, consecuencia del mejoramiento genético en esta especie a través de la selección y/o cruzamientos de hembras de reemplazo y con el uso de sementales de alto mérito genético (Escareño *et al.,* 2013).

Por otra parte, la caprinocultura representa una actividad pecuaria de suma importancia a nivel mundial, nacional y regional ya que los caprinos son catalogados como los mejores rumiantes debido a que: usan la vegetación natural sin competencia con los humanos; utilizan más eficientemente el agua; mantienen la biodiversidad bajo uso de energías no renovables; tienen una alta capacidad de permanencia-resiliencia-sostenibilidad y; favorecen el

mantenimiento de tradiciones, habilidades y conocimientos ancestrales (Navarrete-Molina *et al.,* 2020).

Estacionalidad reproductiva de la cabra

En la mayoría de los ecosistemas del planeta la disponibilidad de vegetación en cantidad y calidad varía de acuerdo a la estación del año; en este sentido, la latitud juega un papel fundamental debido a que entre más alejados estén los hábitats del ecuador, las variaciones por efecto de la estación son más notorias. Debido a lo anterior, podemos definir la estacionalidad reproductiva como uno de los mecanismos de adaptación desarrollados por diversas especies de mamíferos, para restringir la el proceso reproductivo a una época del año con el propósito de asegurar que los periodos de mayor disponibilidad de alimento coincidan con las etapas de mayor exigencia nutricional para las hembras (entre otras, último tercio de gestación, parto y lactancia; Bronson, 1989; Karsch *et al.,* 1984; Lehman *et al.,* 1997; Zerbe *et al.,* 2012).

La estacionalidad reproductiva en las razas caprinas originarias y/o adaptadas a latitudes templadas (> 40° Latitud Norte o Sur) y subtropicales (> 23º < 40º Latitud Norte o Sur) es muy marcada, en comparación a regiones cercanas a la línea ecuatorial (Amoah *et al.,* 1996). A medida que el origen de las razas es más cercano al ecuador (<20° N o S), la estacionalidad reproductiva de las cabras disminuye notablemente (Chemineau *et al.,* 1992).

En las cabras del subtrópico mexicano, específicamente en la Comarca Lagunera (Latitud 26° N), la estación sexual de las hembras multirraciales se presenta de agosto a febrero, y la época anovulatoria se observa de marzo a julio (Carrillo *et al.,* 2010; Contreras-Villarreal *et al.,* 2015; Alvarado-Espino *et al.,* 2016).

Ciclo estral de la cabra

Las hembras caprinas que manifiestan estacionalidad reproductiva son consideradas como poliéstricas estacionales con ovulaciones espontáneas, consecuencia del ciclo estral, el cual definiremos como el lapso entre dos estros, en donde la hembra sufre modificaciones hormonales, anatómicas y de

comportamiento socio-sexual (Bartlewski *et al.,* 2011; Chemineau *et al.,* 1992; Fatet *et al.,* 2011). El intervalo de tiempo del ciclo estral en la cabra varía de 18 a 24 días con un promedio de 21 días (Fatet *et al.,* 2010; Rivera-Lozano *et al*., 2011).

El ciclo estral de la cabra está dividido en dos fases: la fase folicular (proestro y estro), que comprende desde la lisis del cuerpo luteo (luteólisis), crecimiento de los folículos, hasta la ovulación y la fase luteal (metaestro y diestro) que comienza después del proceso ovulatorio y con la formación del CL hasta la luteólisis (Driancourt, 2001).

El proestro tiene una duración promedio de 3 días, y se le denomina periodo de dominancia folicular; debido al efecto que ejerce la FSH sobre los folículos; esta subfase inicia a partir de la regresión del cuerpo lúteo y se caracteriza por una disminución en la secreción de progesterona y por un rápido crecimiento folicular y un incremento en la secreción de estradiol por los folículos (Bartlewski *et al.,* 2011; Medan *et al.,* 2003). El estro es el periodo de receptividad sexual de la cabra y tiene una duración promedio de 3 días, la cual puede variar según la edad, raza, estación del año y el efecto macho (Fatet *et al.,* 2011; Rosa y Bryant, 2003).

Los niveles elevados de E2 secretado por los las estructuras foliculares son las responsables de la aparición del estro y del despliegue de conductas sexuales en la hembra, a la vez que inducen el pico pre-ovulatorio de LH, el cual provoca la ovulación de 30 a 36 horas después del inicio del estro (Bartlewski *et al.,* 2011). El metaestro inicia con la ovulación y tiene una duración de 2 a 5 días, mientras que el diestro tiene una duración promedio de 12 días, y se caracteriza por la presencia del cuerpo lúteo funcional, el cual secreta P4 (Medan et al., 2003). Si es que ocurre la fertilización, el cuerpo lúteo persiste manteniendo elevadas concentraciones plasmáticas de progesterona y por lo tanto la gestación. En caso contrario, el cuerpo lúteo es destruido por acción de la prostaglandina F2α secretada por el utero, la cual disminuye notablemente las concentraciones

plasmáticas de P4 y, por consecuencia, permite nuevamente el inicio del ciclo estral (Driancourt, 2001; Fatet *et al.,* 2011; Figura 1).

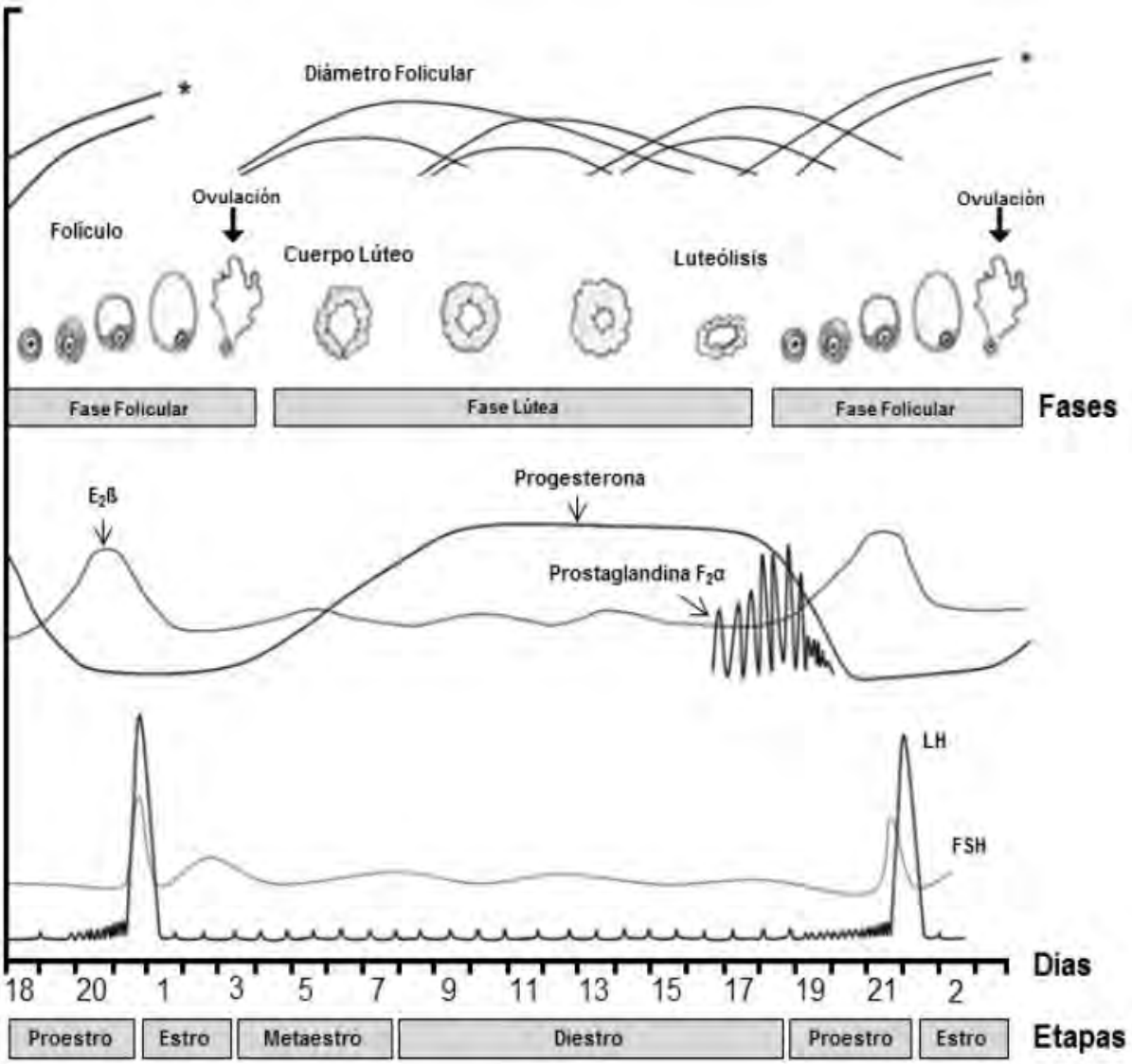

Figura 1. Esquema de los acontecimientos fisiológicos ováricos y endocrinos que se efectúan durante el ciclo estral de la hembra caprina. (Fatet *et al.,* 2011).

Protocolos hormonales para inducir la actividad sexual en las cabras

Los sistemas de producción caprina se enfrentan a un factor limitante consecuencia de la estacionalidad reproductiva que es la estacionalidad en la producción de leche, carne, cabrito y derivados, lo que limita la posibilidad de mayores ingresos a los caprinocultores (Holtz, 2005) y restringe la producción homogénea a lo largo del año. Debido a esto, se han establecido diferentes métodos de control reproductivo, como el uso de protocolos hormonales que permiten romper al anestro de las hembras caprinas y por consecuencia sincronizar el estro y la ovulación. Por ejemplo, los progestágenos son comúnmente utilizados para inducir el estro y la ovulación durante el anestro estacional (Leboeuf *et al.,* 2003), lo que permite el uso de biotecnologías reproductivas como la inseminación artificial a tiempo fijo (IATF), que permite el mejoramiento genético en esta especie.

Los análogos de P4 con más utilidad para sincronizar e inducir el estro son el acetato de fluorogestona (FGA) y el acetato de medroxiprogesterona (MAP) aplicados por medio de esponjas intravaginales (Romano, 2004; Holtz, 2005). La duración de este tipo de tratamientos se ha reducido en base a hallazgos, buscando un menor daño en la salud y la fertilidad de las hembras, como lo prueban los reportes de Viñoles *et al*., 2001; Menchaca y Rubianes, 2004; Rodríguez-Martínez *et al.,* 2013 y Contreras-Villarreal *et al.,* 2015, quienes encontraron efectos positivos del uso de protocolos hormonales con dosis menores de progestágenos y por ende, una menor repercusión negativa en la fertilidad de las hembras.

Gonadotropina coriónica humana (hCG)

La gonadotropina corinica humana es sintetizada por las células trofoblasticas del embrión en la especie humana al periodo cercano al mes de embarazo, y su efecto promario es la estimulación de la sintesis de P4 por el CL, inhibiendo las contracciones del utero, bloqueando la respuesta del sistema inmunologico promoviendo el crecimiento del feto (Cole, 2010). La hCG es una hormona cuya estructura bioquímica es glicoprotéica que integra un conjunto de biomoleculas

que actúan en forma activa. La estructura común de la hCG es producida por la hipofisis a la par con el pico de la hormona luteinizante (Birken *et al.,* 1996). Está compuesta por 244 aminoácidos, con semejanza a las gonadotropinas LH, (70%) y FSH (30%). Por lo cual es utilizada en diferentes especies, además de que su prolongada vida media que permite un estímulo de LH más prolongado (De Rensis *et al.,* 2010). En cabras, su tasa de absorción después de administrarla por vía IM es de 11.6 h, con una vida media de 39.4 h (Saleh *et al.,* 2012).

Como consecuencia del efecto similar a la LH en las células ováricas, la hCG ha sido utilizada en cabras en todo sitios diferentes del planeta para inducir el estro y durante el postparto (Fonseca *et al.*, 2005; Kawate *et al.,* 2002). En años recientes se ha utilizado en la Comarca Lagunera en cabras locales durante el anestro estacional (Alvarado-Espino *et al.,* 2016; Rodríguez-Martínez *et al.,* 2017; Alvarado-Espino *et al.,* 2019) En el primer estudio se utilizaron diferentes dosis de hCG en cabras de raz Alpina, encontrando que con 100 UI de hCG son suficientes para inducir la actividad estral; en el segundo estudio se midió el efecto de dos diferentes rutas de aplicación: intramuscular e intravulvosubmucosal de hCG para inducir al estro, reportando que una inyección de 100 UI de hCG es suficiente para inducir la actividad estral. En el tercer estudio se utilizaron 20 mg de P_4 y 100 UI de hCG en dos sistemas de producción, intensivo y extensivo, con cabras Alpinas y cabras locales, y en diferente época reproductiva; anestro (abril) y en época de transición (junio), la conclusión de los referidos estudios fue que con la utilización de la hCG (100 UI) es suficiente para romper el anestro estacional en la cabras de la Comarca Lagunera.

Otros resultados positvos se han encontrado en diferentes regiones del mundo con el uso de hCG, como lo reportado por Fonseca *et al.,* 2018 y por Côrtes *et al.,* 2020, quienes aplicaron 300 UI de hCG siete días después del estro en cabras lecheras de la raza Toggenburg bajo un sistema intensivo en Minas Gerais (Latitud 21° LS); en otros estudios, el aumento en el área lútea fue seguido por un incremento en los niveles sanguineos de P4. Estudios previos en rumiantes

domésticos comprueban el efecto de la hormona hCG sobre el incremento del área luteal, correlacionado con la concentración de P4 plasmática, encontrando una correlación positiva (Siqueira *et al.,* 2009; Arashiro *et al.,* 2010; Catalano *et al.,* 2012; Figueira *et al.,* 2015; Catalano *et al.,* 2016). La administración de hCG el día 4 después del IATF aumentó constantemente la concentración plasmática de P4 (Fonseca *et al.,* 2018), lo que confirma los resultados de estudios anteriores, que habían informado que la aplicación de hCG en los días 6 o 9 (Ishida *et al.,* 1999), 3, 4 o 5 (Fukui *et al.,* 2001) o 4, 7 y 10 post estro (Lankford *et al.,* 2010) inducen la sintesis de cuerpos lúteos (CL) accesorios y aumentaron la concentración plasmática de P4 en comparación con los controles. En este sentido, los resultados reportados por Fernandez *et al.,* 2018 y Fernandez *et al.,* 2019, comprueban el efecto positivo de la hCG sobre la formación de CL accesorios y su correlación con la producción de P4 plasmática, en ovejas de la raza Merino al Norte de la Patagonia.

Reconocimiento temprano de la gestación en la cabra.

En los rumiantes domésticos el establecimiento de la gestación requiere de señalización del reconocimiento de la misma, seguida del proceso de implantación y posteriormente de la placentación (Hansen *et al.,* 2017). El alargamiento del conceptus (embrión o feto y membranas embrionarias), es un episodio clave para la producción de interferón tau (IFNT), y por ende el reconocimiento temprano de la gestación e implantación embrionaria (Gray *et al.,* 2002; Hansen *et al.,* 2017). Derivado del conceptus, el interferón-tau interrumpe la vía de transducción de señales que regula la liberación de prostaglandina F2α, principal producto luteolítico en ovejas, vacas y cabras (Al-Samawi *et al.,* 2021).

El reconocimiento materno del embrión se considera uno de los acontecimientos de más alta relevancia en el proceso reproductivo. Está influenciado por gran cantidad de eventos celulares y endocrinologicos entre el embrión, el utero y el CL. (Yasser *et al.,* 2010). El interferón (INT-t) interviene en los eventos luteotrópicos de función y estructura, priorizando la sintesis de P4 y la forma de la estructura celular que constituyen el CL.

El reconocimiento temprano de la gestación en la cabra como en otros rumiantes está regulado por los interferones, los cuales actúan sobre el endometrio de manera paracrina para prevenir la luteólisis, manteniendo así el CL y la producción de P4 (Roberts, 2007); además de ser la señal para el reconocimiento temprano de la gestación, los IFN´s juegan otras funciones en la gestación temprana en rumiantes, entre ellas se incluyen la protección del feto o del útero contra la infección viral y la modulación de la respuesta inmune materna para la tolerancia fetal. Los IFN´s se originan en el conceptus y no en el endometrio en las cabras, observando secreción de IFN del día 10 al 21 (día 0 = apareamiento) con niveles máximos entre los días 16 y 18. Probablemente entre los días 22 y 23 la síntesis de IFN ha cesado (Martal *et al.,* 1990). El tamaño del blastocisto es un factor que determina la cantidad de IFN producido que puede no coincidir necesariamente con la cantidad de IFN expresado por el blastocisto.

Implantación embrionaria en la cabra

El proceso de implantación embrionaria en los mamíferos es un evento fundamental en fisiología de la reproduccion y es producto de una serie de eventos tisulares complejos que depende de una intrincada diafonía entre el ovario, el útero y el conceptus; la tasa más alta de pérdidas gestacionales ocurre durante el período de peri-implantación (Meza-Herrera *et al.,* 2019). Cuando ocurre este proceso fisiológico frecuentemente ocurren modificaciones a nivel molecular en los animales de interés zootécnico así como silvestres y hoy dia no se sabe que determina la aparición de estas disturbios molecualres. La implantación requiere un par de factores claves y fundamentales, un blastocisto con capacidad de llevar a cabo la implantacion y, de manera similar es necesario un endometrio capaz de llevar a cabo el proceso de recepción del blastocisto (Vigano *et al.,* 2003; Salamonsen *et al.,* 2002; Góngora, 2002).

El ovocito fecundado lleva a cabo un movimiento en dirección al zona inferior del oviducto (ampolla) en sincronía inicia el proceso biológico de mitosis. Posteriormente el ser recientemente formado se mantiene de manera libre en el útero hasta que ocurre la unión con la pared uterina, este proceso ocurre en el

intervalo gestacional de los días 20 y 25 posterior a la fecundación (Harvey, *et al.,* 1995). Este mismo autor menciona que "el éxito" correspondiente al proceso de la implantación es dependiente de la sinergia efectuada entre madre-embrión inducidos en el útero, el cual está estrechamente relacionado con la síntesis y función de las hormonas E2 y P4.

El proceso fisiológico de implantación embrionaria está influenciado por varios factores que limitan que se lleve a cabo o no, los cuales son, carencia de rechazo del sistema inmune, salud en la madre a nivel uterino y, alto valor genético en el embrión (Hernández *et al.,* 2002).

Una vez fertilizado, el cigoto baja por el oviducto y alcanza el útero alrededor del día 4 post-coito. Alrededor del día 6 pc se produce la evolución a blastocisto, constituido por una cavidad rodeada por una monocapa celular. Despues (días 8-9 pc), se rompe la zona pelúcida y el blastocisto eclosiona exponiendo la superficie del trofoblasto al medio del utero (Roberts *et al.,* 2008). La implantación embrionaia se inicia con la fase de aposición (días 12-15 pc), que consiste en la unión inestable del embrión al endometrio (Bazer *et al.,* 2009). La implantación ocurre por unión firme del embrión al epitelio endometrial superficial alrededor del día 16 post coito iniciando la síntesis a una placenta sinepiteliocorial no invasiva (Brooks *et al.,* 2014).

En las cabras, el cuerpo lúteo es la única fuente de secreción de P4, ya que la placenta no la produce durante la gestación (Raheem, 2015). Este escenario fisiológico puede generar cambios en los niveles críticos de ciertas hormonas que pueden comprometer la correcta implantación del embrión (Bazer *et al.,* 2010; Bazer *et al.,* 2018).

El uso reciente de protocolos hormonales para incrementar las tasas de implantación embrionaria en rumiantes han ofrecido resultados favorables; por ejemplo, está comprobado que el uso de la hCG incrementa los niveles de P4, mejora la supervivencia embrionaria y reduce las pérdidas de embriones en rumiantes (Schmitt *et al.,* 1996; Rostami *et al.,* 2016). Además, en otros estudios realizados en ovinos (Nephew *et al.,* 1994; Khan *et al.,* 2007;Khan *et al.,* 2009)

se encontró diferencia en la variable de implantación embrionaria cuando se administró 100 UI de hCG en ovejas. Esto concuerda con un estudio anterior realizado en cabras de la raza Florida manejadas bajo sistema semi-extensivo, en el que se obtuvo una menor tasa de implantación que en las mantenidas en sistema intensivo (35.1% vs 51.1%, respectivamente; P <0.0001). Es probable que la menor tasa de preñez observada en cabras bajo este sistema de manejo extensivo rural se deba a una baja respuesta estral u ovulatoria al protocolo de sincronización, o una pérdida temprana de embriones después de la IATF (Samir *et al.,* 2016).

Principales causas y consecuencias de las pérdidas fetales en cabras

En las cabras manejadas bajo sistema extensivo sedentario, predominante en regiones áridas, las pérdidas de gestaciones en los primeros tres meses son frecuentes y representan la primera falla de la reproduccion en las cabras en estos sistemas de producción, donde muy frecuentemente se presenta una estacionalidad en la disponibilidad en cuanto a calidad y cantidad de la vegetación nativa a través del proceso gestacional. Además los abortos en estas áreas agroecológicas se presentan en más del 50% de las hembras caprinas en gestacion (Mellado y Pastor, 2006). Las hembras que carecen de cornamenta, y en marasmo nutricional, aunado a gestaciones múltiples y falta de minerales Mg, Cu, y Se son de importancia y representan un peligro, y como consecuencia abortos en esta especie (Mellado y Pastor 2006).

En las cabras como en las ovejas, la mortalidad embrionaria temprana se presenta entre los días 2 y 15 de la preñez, como consecuencia de una sub o sobrealimentación de la madre. Un cambio brusco en la dieta causa un rápido cambio en la fermentación ruminal y altera el pH uterino, el cual afecta la sobrevivencia del embrión (Bazer et al., 2010). Además, se ha encontrado como causa importante un desequilibrio hormonal, específicamente la disminución en los niveles de P4, que se traduce en una mayor tasa de pérdidas embrionarias tempranas.

El fracaso en la gestación está dado por pérdidas o muertes embrionarias las cuales se dividen en precoces y tardías. Las precoces se consideran entre la fecundación y los primeros 20 días, mientras las segundas tienen efecto entre los 21 y los 35-40 días aproximadamente (Gray *et al.,* 2002; Bazer et al., 2009). Las muertes embrionarias precoces representan el mayor porcentaje perdidas gestacionales (15-30 % de los ovocitos liberados), mientras que las tardías ya consideradas como fetos son de menor magnitud (5-7 %). Por otra parte, el 20% de la mortalidad embrionaria se atribuye a insuficiencia de la función lútea y se concentra en los 15 días después del proceso de fertilización del óvulo (Bazer *et al.,* 2018) durante el período de concepción-elongación.

En cabras como en otros rumiantes, la consecuencia principal de las pérdidas gestacionales son la disminución en la producción de leche, carne, cabrito y derivados, lo que disminuye los ingresos a los caprinocultores (Mellado *et al.,* 2004), así mismo, la capacidad reproductiva de las hembras con estas fallas se ve reducida y limita la posibilidad de generar reemplazos dentro de su sistema de producción.

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ESTUDIO 1

ARTICULO 1: Luteogenesis and embryo implantation are enhanced by exogenous hCG in goats subjected to an out-of-season fixed-time artificial insemination protocol.

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Luteogenesis and Embryo Implantation Are Enhanced by Exogenous hCG in Goats Subjected to an Out-of-Season Fixed-Time Artificial Insemination Protocol

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Simple Summary: In temperate and subtropical ecosystems, goats are classified as seasonal-polyestrous, generating a seasonality in their products (i.e., milk, meat). In order to abolish or reduce this reproductive seasonality, exogenous hormones, such as human chorionic gonadotropin (hCG), have been used to stimulate the development of ovarian follicles, triggering ovulation, corpus luteum formation, while increasing serum progesterone levels, fetal growth, prolificacy, and kidding rate. We tested the possible effect of two doses of hCG (i.e., 100 vs. 300 IU) in the improvement of the luteal function and embryo implantation in anovulatory goats subjected to an estrus induction protocol, and then, to a fixed-time artificial insemination protocol (FTAI). The highest values for fecundity rate, corpus luteum area, as well as embryonic efficiency index 1, were favored in goats treated with 300 IU hCG 14 days post-artificial fertilization. The embryonic efficiency and the index embryonic implantation efficiency were favored in goats treated with 300 IU hCG 7 and 14 days post-artificial insemination. This work contributes to explore the most suitable use of exogenous hormones that favor the out-of-season reproductive outcomes in goats and to boost the embryo implantation efficiency.

Abstract: The aim of this study was to evaluate the possible effect of two doses of hCG (100 and 300 IU) applied at two different times (7 and 14 d) after a fixed-time artificial insemination protocol (FTAI) upon some variables involved in the embryonic implantation rate in goats during the natural deep anestrous season (April, 25° north). The experimental units considered crossbred, multiparous, anovulatory goats (n = 69, Alpine, Saanen, Nubian x Criollo), with average body weight (43.6 ± 5.7 kg) and body condition score (1.86 ± 0.28 units) located in northern–semiarid Mexico (25° N, 103° W). Once the goat's anestrus status was confirmed, goats were subjected to an estrus induction protocol. Upon estrus induction confirmation, goats (n = 61) were subjected to a FTAI procedure. Immediately after the FTAI, the goats were randomly distributed to five experimental groups: (1). G100-7 (n = 13) 100 IU, hCG 7 d post-FTAI, (2). G100-14 (n = 12) 100 IU hCG, 14 d post-FTAI, (3). G300-7 (n = 12) 300 IU, hCG, 7 d post-FTAI, (4). G300-14 (n = 12) 300 IU hCG 14 d post-FTAI, and (5). Control group, CONT (n = 12) 0.5 mL saline, 7 and 14 d post-FTAI. The response variables conception rate (39.36 ± 0.23), fertility rate (27.96%), prolificacy rate (1.1 ± 0.29 kids), ovulation rate (0.74 ± 0.20 corpus luteum) corpus luteum diameter (10.15 ± 0.59 mm), embryo number (1.58 ± 0.20), and embryo implantation rate (48.96%), did not differ between treatments. However, while the variables fecundity rate (67%), embryo efficiency index-1 (33.99 ± 0.20%), and embryo efficiency index-2 (27.94 ± 0.30%) were favored by the G300-14 treatment, the corpus luteum

area was favored ($p < 0.05$) by both G300-7 (113.30 ± 0.19 mm^2) and G300-14 (103.04 ± 0.17 mm^2). Such reproductive strategy emerges as an interesting approach, not only to enhance the out-of-season reproductive outcomes, but also to boost one of the main rulers defining the global reproductive efficiency of a heard, namely, the embryo implantation efficiency.

Keywords: goats; reproduction; seasonality; FTAI; reproductive efficiency; embryo implantation

1. Introduction

Goat production is an important activity that has benefited human development since the dawn of our civilization; a significant percentage of the rural population in marginal ecosystems, from an ecological and social perspective, depends on this activity for the sustainability of their livelihoods [1,2]. Mexico has an inventory close to 9.0 million goats, highlighting The Comarca Lagunera (TCL), an agroecological region located in the arid north of Mexico, with respect to the levels of production and goat census at the national level with about 400,000 goats [3]; TCL ranks first in goat milk production in Mexico [2,4,5]. In recent years, a reduction in the goat inventory has occurred in TCL, although accompanied by increases in milk production [5]. This positive trend in the efficiency of dairy production suggests a greater selection pressure, both in replacement females, and in the selection of sires, with high genetic merit for dairy production, mainly Saanen and Alpine [2,5].

In this regard, both in temperate and subtropical ecosystems, goats are classified as seasonal-polyestrous; this seasonal reproduction in turn generates a seasonality in goat products such as meat, milk and their derivatives [6–8]. In TCL, the multiracial local goats show a period of deep anestrus from March to May; the reproductive season begins in August, ending in February [8–11]. In order to abolish or reduce this reproductive seasonality, exogenous hormones, such as human chorionic gonadotropin (hCG) with different doses (i.e., 50, 100, and 300 IU), have been used in goats. For instance, at the onset of the breeding season (i.e., 0, 50, 100, and 300 IU; [11]), 100 IU using different administration routes [12], as well as during the seasonal anestrous [13], and 100 IU either during the deep anestrous, and the transition from anestrous to the breeding season [14]. The administration of hCG in anestrus goats stimulates the development of ovarian follicles, triggering ovulation, and the formation of the corpus luteum [11,15]. In the same way, hCG has been used to increase not only plasma progesterone levels, but prolificacy, fetal growth, and kidding percentage, by using 150 and 300 IU at the onset of the breeding season [16]. The critical period of gestation is directly linked to the maternal recognition of pregnancy process (MRPP), which initiates because of the presence of the conceptus in the uterus; it activates an antiluteolytic mechanism designed to protect the corpus luteum [17,18]. Certainly, by safeguarding luteogenesis, the synthesis and release of progesterone, necessary to initiate embryo implantation and establish gestation, is throughout the MRPP; the main antiluteolytic signal released by conceptus–trophoblast cells is the interferon tau (INFτ) [17–20].

In goats, gestation is maintained by progesterone secreted by the corpus luteum [21]; any luteal dysfunction generating low progesterone concentrations during the early stages of pregnancy will seriously compromise the embryo implantation process [22,23]. Indeed, about 20% of embryonic mortality attributed to luteal failure is concentrated during the 15 days after the ovum fertilization process [22,24], particularly during the period of conceptus elongation, which occurs prior to the MRPP, ending with embryo implantation [23]. Certainly, an inadequate luteal function is one of the most relevant causes of pregnancy failure in goats [25,26]. Building on the above findings, we tested the hypothesis that a low administration of hCG after fixed-time artificial insemination (FTAI), positively affects the MRPP in goats during the anestrous period, improving luteogenesis and embryonic implantation rate, in anovulatory goats subjected to an estrus induction protocol, and subsequently exposed to FTAI, managed under extensive conditions. Moreover, a positive

effect of hCG upon other reproductive response variables should be expected; our study aims to elucidate such inquiries.

2. Materials and Methods

2.1. General

All of the experimental procedures, methods, and handling of the experimental test units used in this study complied with the guidelines for the ethical use, care, and well-being of research animals at the international [27] and national [28] levels, with institutional approval reference number UAAAN-UL-18-3059.

2.2. Location, and Environmental Conditions

The study was carried out in the Ejido Venecia, Gómez Palacio, Durango, Mexico (25° 47′ NL, 103° 21′ WL, altitude = 1111 m). Rainfall occurs from June to September with an annual average of 266 mm (range 163 to 440 mm). This region has a dry climate with an average annual temperature of 21 °C varying from 37 °C (May–August) to 0 °C in winter [29]. The goats were fed under the extensive sedentary grazing system predominant in the Comarca Lagunera, consuming the native vegetation, such as Buffelgrass (*Cenchrus ciliare*), Bermuda grass (*Cynodon dactylon*), Navajita (*Bouteloua* spp.), Johnson (*Sorghum halepense*), Chamizo (*Atriplex canescens*), as well as Mesquite sprouts and fruits (*Prosopis glandulosa*), Huizache (*Acacia farnesiana*), shrubs, and eventually crop residues, such as sorghum, melon, cotton watermelon, forage oats [30]. The goats went out to grazing at 10:00 a.m. and returned to the corral at 06:00 p.m. The goats were hand-milked once a day at 07:00 a.m. All goats were subcutaneously dewormed (Ivermectin 1%, Baymec, Bayer, Mexico City, Mexico) and also received doses of vitamin A (500,000 IU), D3 (75,000 IU), E (50 mg) (Vigantol: ADE + Selenium, 250 mL, Zapopan, Jalisco, Mexico), one-month prior the onset of the study; water, shades, and mineral salts (17% P, 3% Mg, 5% Ca, and 75% NaCL) were freely available. All of the goats received daily individual nutritional supplementation (10:00 a.m.) during 10 d pre- and 10 d post-FTAI; the supplement consisted of 100 g of rolled corn (8.6% CP) and 500 g of alfalfa hay (18% CP), as suggested by [31].

2.3. Animals and Their Management

2.3.1. Female Goats: Confirmation of the Anovulatory Status, Estrus Induction Protocol, and Estrus Detection with Aproned Males

From a commercial crossbred goat herd (n = 155; Alpine, Saanen, Nubian x Criollo), a total of 69 multiparous, anovulatory goats, with 43.6 ± 5.7 kg live weight, 1.86 ± 129 0.28 units body condition score, and 2–4 kidding were selected and identified with earrings for their best management during the experimental period. Anovulation was determined in March by means of an ultrasound fitted to a 7.5 MHz transrectal transducer (Aloka SSD-500, Richmond, Canada). Each goat underwent an ultrasound on days 14 and 7 prior to the application of progesterone (P4), in order to confirm the absence of corpora lutea in both ovaries. Once anovulation was confirmed, the goats were subjected to an estrus induction protocol. On day -1, all females received 20 mg of P4 (Progesvit®, Brovel, i.m., Irapuato, Guanajuato, Mexico) in order to avoid the presence of short cycles. The following day, day zero (0 d), the goats received 200 IU of hCG (Chorulon®, Intervet, Mexico City, Mexico.) i.m., in order to stimulate the formation of the antrum in the ovarian follicles in advanced stages of development, and promote, in turn, ovulation. Both P4 and hCG were applied around 08:00 h of the respective day. The detection of estrus considered the use of three sexually active adult males provided with aprons to prevent them from copulating with the female. The goat was considered in standing estrus when it remained immobile when the male rode it. Immediately, the female was removed from the pen for the male to continue detecting heat. From the initial 69 goats exposed to the estrus induction protocol, only 61 were declared in estrus based on their interaction with the aproned males.

2.3.2. Male Goats: Setting Bucks for Semen Extraction and Semen Quality Assessment

Male goats (n = 3; Granadina breed; 2.5 years old) were used; this breed shows an optimal level of adaptability and productivity in free-ranging production systems [32]. Besides, this breed displays a reduced reproductive seasonality; females are considered as continuous polyestrous, presenting kidding all-year-around without the need for the use of exogenous hormones [33,34]. Besides, bucks have a lessened period of sexual rest, nonetheless, in our study, in order to induce an intense sexual activity and libido, the bucks used for semen collection, received an application of 50 mg of testosterone (T) (Testosterone-50, steroidal androgenic, i.m., Lab Brovel, Mexico City, Mexico) every third day for three weeks' prior to the onset of the trial as previously suggested in this geographical area [35]. Males received a daily nutritional supplementation 60 d prior semen collection, based on 2 kg alfalfa hay per male (2.3 Mcal/kg, 18% PC/kg DM) plus 600 g of a commercial concentrate (1.7 Mcal/kg, 14% PC/kg DM) as previously recommended [31] in order to enhance both metabolic and physiological outputs [36]. By the end of April, the semen was extracted with the use of an artificial vagina (Walmur, Montevideo, Uruguay); the ejaculate was evaluated macro and microscopically, considering motility, viability, and sperm concentration. For the FTAI process, only ejaculates with the following characteristics were used: volume of $\geq$0.5 mL, sperm mass motility of $\geq$3 (scale 0–5), sperm concentration $\geq 2500 \times 10^6$ cells/mL, and progressive motility $\geq$70%. Once the semen quality of the three bucks was confirmed, in order to avoid any confounded sire effect upon the embryo implantation rate, the collected samples of semen were mixed, to obtain a final composite semen sample. Thereafter, the semen was diluted (Optidyl^{TM}, Cryo-Vet, Leon Guanajuato, Mexico) to obtain a final concentration of 200×10^6 per dose (0.2 mL) and was kept in plastic straws in refrigeration (4 °C); prior to the FTAI, another microscopic analysis was implemented to verify the quality of the diluted semen.

2.4. Fixed-Time Artificial Insemination (FTAI)

Immediately after the estrus induction protocol (i.e., 20 mg P4 and 200 IU hCG), and once the estrus status was confirmed, all goats (n = 61), in late April, were exposed to the FTAI protocol. Briefly, once the sperm quality was re-evaluated, the diluted semen was transferred to a container with water at 30 °C. The FTAI procedure was carried out in the handling pen, using a vaginoscope for veterinary use (Walmur-Veterinary Instrument, Montevideo, Uruguay) equipped with a light source. The semen was deposited in the pericervical area 58 h after the application of 200 UI of hCG, then, the next day at 09:00 h (72 h after the application of 200 UI hCG), a second FTAI was carried out; that is, all females were subjected to the FTAI protocol twice; 58 and 72 h after applying the 200 IU of hCG.

2.5. Conformation of the Experimental Groups: The Use of hCG Post-FTAI to Enhance the Embryo Implantation Rate

Once all the goats were artificially inseminated (FTAI, n = 61), they were randomly distributed to five experimental treatments considering two doses of hCG (100 and 300 IU, Chorulon®, Intervet, Mexico City, Mexico) and two application times (7 and 14 d post-FTAI), plus the Control group. Therefore, the experimental groups were conformed as follows: (1). G100-7 (n = 13) 100 IU, hCG 7 d post-FTAI, (2). G100-14 (n = 12) 100 IU hCG, 14 d post-FTAI, (3). G300-7 (n = 12) 300 IU, hCG, 7 d post-FTAI, (4). G300-14 (n = 12) 300 IU hCG 14 d post-FTAI, and (5). Control group, CONT (n = 12) 0.5 mL saline 7 and 14 d post-FTAI. In all the experimental groups, the hCG was applied i.m. between 08:00 and 09:00 on the neck, either 7 or 14 days post-FTAI. Therefore, the possible effect of these five experimental treatments upon embryo implantation rate, luteogenesis as well as other reproductive variables, was evaluated (Figure 1).

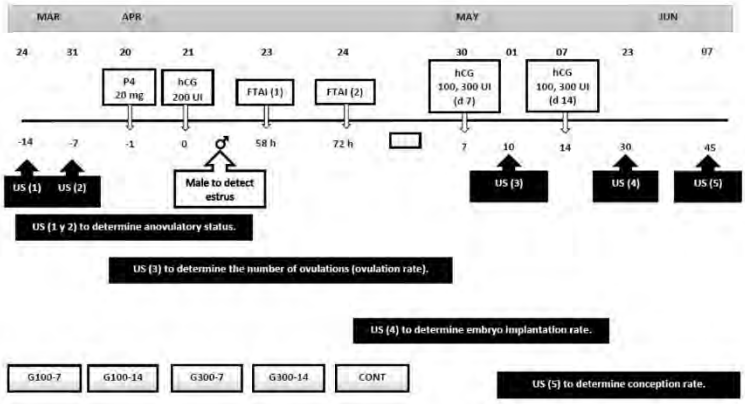

Figure 1. Schematic representation of the experimental protocol. The application of hCG (100 and 300 UI) was on day 7 and 14 post-FTAI. Ultrasound (US) was performed to determine anovulatory status, the number of ovulations (ovulation rate), the embryo implantation rate, and conception rate.

2.6. Response Variables

2.6.1. Body Weight, Body Condition Score, and Estrus Induction

Body weight (BW) and body condition score (BCS) were recorded at the beginning of the experimental period and when performing the corresponding ultrasounds. To determine BW, an electronic scale with a capacity of 250 kg and an accuracy of 50 g was used (Torrey 110v/220v, Digital Industrial Scale, Jalisco, Mexico). Moreover, BCS was determined by one experienced technician as previously described [37], considering a scale from 1 (very thin) to 4 (very fat).

2.6.2. Ovulation Percentage, Ovulatory Rate, and Luteogenesis

The percentage of ovulating females was determined on days 0 and 10 after the estrus induction protocol by means of a transrectal ultrasonography evaluation (Aloka SSD-500, Richmond, Canada) using a 7.5 MHz linear probe. The ovulatory rate was determined through ultrasonography on day 10 after the FTAI; the luteal area was determined considering the diameter of the corpora lutea observed in the ultrasonography.

2.6.3. Embryo Implantation Rate, and Other Reproductive Variables

On d-30 post-FTAI, the embryo implantation rate was determined through transrectal ultrasonography using color Doppler equipment (Chison ECO-5, with 12 inch probe); at this time, the embryonic implantation process should have occurred in the uterus. Other reproductive response variables collected within treatment were conception rate: determined on day 45 post-FTAI, considering the pregnant goats/inseminated goats, fecundity rate: considering the number of fetuses per inseminated goat, fertility rate: the number of pregnant females that gave birth, prolificacy rate: determined at parturition and considering the number of kids born per pregnant female. Moreover, two indices were developed in order to ponder the success of the embryo implantation rate with respect to both conception rate and fecundity rate: Embryo Efficiency Index 1: (embryo implantation rate) (conception rate/100), and Embryo Efficiency Index 2: (embryo implantation rate) (fecundity rate/100).

2.7. Statistical Analyses

A first linear model was developed to evaluate the possible relationship of hCG dose (i.e., 100 vs. 300) and period of administration (i.e., 7 vs. 14 d post-FTAI) with respect to

body weight (BW, kg), corpus luteum diameter (CLD, mm), corpus luteum area (CLA, mm^2). Regarding percentage and counts variables body condition score (BCS, units), estrus induction (EI, %), conception rate (CR, %), fertility rate (FR, %) prolificacy rate (PR, %), fecundity rate (FC, %), ovulation rate (OR, units), embryo number (EN, units), embryo implantation rate (EIR, %), embryo efficiency index-1(EEI-1, %) and embryo efficiency index-2 (EEI-2, %), since they do not fit normal distribution, they were log^{10} transformed prior to ANOVA to overcome skewness. Least-squares means and standard errors for each experimental treatment were computed; multiple mean comparisons were performed through the Fisher's LSD–LSMEANS option of the PROC GLM of SAS. Since all the experimental treatments were individually evaluated, each goat within the experimental group was defined as the experimental unit; treatment differences were accepted if $p < 0.05$. All the analyses were computed through the procedures of SAS (SAS Inst. Inc. version 9.4, Cary, NC, USA).

3. Results

3.1. Body Weight, Body Condition Score, and Estrus Induction

No differences ($p > 0.05$) regarding body weight (BW, 45.6 ± 1.21 kg), body condition score (BCS, 1.88 ± 0.08 units), or estrus induction (EI, 88.41% 61/69) occurred among experimental groups (Table 1). Prior to the estrus induction protocol, the ultrasound scanning confirmed that 69/69 female goats were anestrous previous to the FTAI protocol.

Table 1. Least-square means ± standard error for body weight (BW), body condition score (BCS), and estrus induction (EI) according to the experimental treatment to be exposed after to the fixed time artificial insemination protocol, in multiracial, multiparous, and anovulatory goats (n = 61, Alpine, Saanen, Nubian x Criollo) managed under extensive conditions in Northern Mexico (April, 25° N) [1].

Variables	G100-7 (n = 13)	G100-14 (n = 12)	G300-7 (n = 12)	G300-14 (n = 12)	CONT (n = 12)	*p* Value
BW, (kg)	45.5 ± 0.80	45.8 ± 1.2	45.9 ± 0.82	45.2 ± 1.50	45.6 ± 1.71	0.99
BCS, (units)	1.9 ± 0.09	2.0 0.07	1.8 ± 0.07	1.9 ± 0.11	1.8 ± 0.07	0.62
EI, (n, %)	13/14 (92.8)	12/15 (80.0)	12/13 (92.3)	12/13 (92.3)	(12/14) 85.71	0.64

[1] No differences ($p > 0.05$) for any variable occurred among experimental groups.

3.2. Conception Rate, Fertility Rate, Prolificacy Rate, and Fecundity Rate

As observed in Table 2, no differences ($p > 0.05$) regarding conception rate, fertility rate, and prolificacy rate occurred across experimental groups when considering two doses of hCG (100 and 300 IU) applied at two different periods of time (7 and 14 d), along with the Control group. Respective averages for the response variables were (39.36 ± 0.23%, 27.96%, 1.1 ± 0.29 kids). Interestingly, however, the phenotypic expression of fecundity rate differed ($p < 0.05$) among the experimental groups, favoring the G300-14 goats.

Table 2. Least-square means ± standard error for conception rate (CR), fertility rate (FR) prolificacy rate (PR), and fecundity rate (FC) according to the experimental treatment considering two doses of hCG (100 and 300 IU) and applied at two different times (7 and 14 d), and the Control group (CONT) after a fixed time artificial insemination protocol in multiracial, multiparous, and anovulatory goats (n = 61, Alpine, Saanen, Nubian x Criollo) managed under extensive conditions in Northern Mexico (April, 25° N).

Variables	G100-7 (n = 13)	G100-14 (n = 12)	G300-7 (n = 12)	G300-14 (n = 12)	CONT (n = 12)	*p* Value
CR (n, %)	5/13 (38.5 ± 0.15)	5/12 (41.7 ± 0.28)	3/12 (25 ± 0.22)	7/12 (58.3 ± 0.20)	4/12 (33.3± 0.30)	0.54
FR (n, %)	3/13 (23.1)	3/12 (25)	3/12 (25)	6/12 (50)	2/12 (16.7)	0.71
PR (n)	1.7 ± 0.29	0.6 ± 0.33	1.3 ± 0.33	1.4 ± 0.21	0.5 ± 0.29	0.53
FC (n, %)	5/13 (38) [b]	2/12 (17) [b]	4/12 (33) [b]	8/12 (67) [a]	2/12 (17) [b]	0.06

[a,b] Response variables with different superscripts within lines, differ ($p > 0.05$).

3.3. Ovulation Rate, Corpus Luteum Area and Diameter, Embryo Number, Embryo Implantation Rate, and Embryo Efficiency Indexes

No differences ($p > 0.05$) among treatments occurred for the response variables ovulation rate (0.74 ± 0.20 units), corpus luteum diameter (10.15 ± 0.59 mm), embryo number (1.58 ± 0.20), and embryo implantation rate (48.96%). In contrast, the largest corpus luteum area was favored ($p < 0.05$) by the G300 group, irrespective of the time of application, regarding the G100-7, G100-14, and CONT, with corresponding values of 113.3, 103.0 vs. 72.7, 79.3, and 45.9 mm (Table 3). Moreover, while no differences ($p > 0.05$) occurred among the G100-7, G100-14, G300-7, and CONT treatment groups neither for EEI1 nor EEI2, the largest EEI1 was favored ($p < 0.05$) by the G300-14 group while the EE2 was favored by both the G300-7 and G300 14 ($p < 0.05$).

Table 3. Least-square means ± standard error for ovulation rate (OVR), corpus luteum diameter (CLD), corpus luteum area (CLA), embryo number (EN), embryo implantation rate (EIR), embryo efficiency index-1(EEI-1) and embryo efficiency index-2 (EEI-2), according to the experimental treatment considering two doses of hCG (100 and 300 IU), and applied at two different times (7 and 14 d), and the Control group (CONT) after a fixed time artificial insemination protocol in multiracial, multiparous, and anovulatory goats (n = 61, Alpine, Saanen, Nubian x Criollo) managed under extensive conditions in Northern Mexico (April, 25° N).

Variables	G100-7 (n = 13)	G100-14 (n = 12)	G300-7 (n = 12)	G300-14 (n = 12)	CONT (n = 12)	*p* Value
OVR *n*	0.61 ± 0.17	0.75 ± 0.22	0.58 ± 0.19	1.27 ± 0.17	0.58 ± 0.23	0.3
CLD (mm)	9.54 ± 0.48	9.91 ± 0.77	11.88 ± 0.71	11.22 ± 0.47	8.21 ± 0.51	0.10
CLA (mm^2)	72.73 ± 0.18 ^{b}	79.37 ± 0.22 ^{b}	113.30 ± 0.19 ^{a}	103.04 ± 0.17 ^{a}	45.96 ± 0.19 ^{b}	0.02
EN *n*	1.75 ± 0.13 ^{a}	1.40 ± 0.24	1.67 ± 0.21	1.57 ± 0.20	1.50 ± 0.24	0.78
EIR *n* (%)	8/13 (61.5)	5/12 (41.7)	6/12 (50)	7/12 (58.3)	4/12 (33.3)	0.61
EEI1 (%) ^{1}	23.68 ± 0.12 ^{b}	17.39 ± 0.24 ^{b}	12.5 ± 0.21 ^{b}	33.99 ± 0.20 ^{a}	11.09 ± 0.24 ^{b}	0.02
EEI2 (%) ^{2}	19.00 ± 0.37 ^{b}	9.91 ± 0.20 ^{b}	20.30 ± 0.25 ^{a}	27.94 ± 0.30 ^{a}	5.66 ± 0.18 ^{b}	0.02

Different letters between columns show difference ($p > 0.05$). Data are presented as mean ± standard error of the mean.[1] Embryo Efficiency Index 1 = (implantation rate) (conception rate/100). [2] Embryo Efficiency Index 2 = (implantation rate) (fecundity rate/100).

4. Discussion

The results obtained in this study do not support our working hypothesis, which proposed that a low dose of hCG (i.e., 100 vs. 300 IU) would be effective to improve luteal function and embryo implantation in anovulatory goats subjected to an estrous induction protocol and, subsequently, to a fixed-time artificial insemination (FTAI) protocol. Indeed, the highest values for the response variables: fecundity rate (FC), corpus luteum area (CLA), embryonic efficiency index 1 (EEI1) was favored by the G300-14 group, while the embryonic efficiency index 2 (EEI2) was favored by both the G300-7 and G300 14 groups. That is, a high dose of hCG administered 14 d after the FTAI, improved the embryonic implantation rate. In this regard, hCG not only has a structure equivalent to LH, with 70% similarity, and a longer half-life (i.e., 39 h vs. 6 h), it also has 30% similarity with FSH, which favors ovarian stimulation at the level of theca cells and FSH receptors in granulosa cells, while both positively influence the critical period of early pregnancy; the embryo implantation process [38]. The present study contributes to a better understanding regarding the use of exogenous hormones that enhance the reproductive performance of previously anovulatory females, being an interesting alternative strategy to reduce reproductive seasonality and improve the embryo implantation rate.

At the beginning of the study, the state of anestrus was confirmed in 100% of the goats (69/69), subsequently, 88.41% (61/69) of them responded positively to the estrus induction protocol. Such trend coincides with other studies evaluating the use of 20 mg of P4 and diverse doses of hCG in both intensive and extensive production systems, in Alpine goats and Crossbred goats, as well as in the deep anestrous (April) as in the time of transition to estrus (June), concluding that this protocol was able to break the seasonal anestrus in more than 90% of goats [11,13,15,16]. Another interesting aspect in our study is that both BW

and BCS did not differ among experimental groups in April, with similar values to those reported in goats managed under an extensive sedentary system in northern Mexico [39]. They also reported that after the dry season (i.e., winter) the BW of goats is significantly decreased under these marginal production systems, generating embryonic or fetal losses in the early stages of gestation.

Regarding the conception rate, fertility rate, and prolificacy rate, they did not differ among experimental groups; the obtained values were higher than those formerly reported [40]. A myriad of environmental, genetic, metabolic, endocrine, physiological, sanitary, and even tissue-related factors, among others, which can be involved in the differences observed among studies. Moreover, all the experimental groups exhibited embryonic losses, so that fertility and the prolificacy rate decreased, coinciding with a previous study [39]. Nonetheless, in our study, the fecundity rate was higher in the G300-14 group, that is, a greater number of fetuses was observed with respect to the number of inseminated goats in that group, coinciding with a study which evaluated the use of different doses of GnRH and hCG four days post-insemination in Merino ewes, in relation to fertility and pregnancy rate [41]. Concerning gestational losses, an incidence of 70% of abortions has been reported in crossbred goats under extensive systems in the arid north of Mexico, affecting the global prolificacy of the herds [25]. In goats, the corpus luteum is the only source of progesterone production since the placenta does not produce it during pregnancy [42]; P4, in turn, influences the synthesis and action of both interferon-tau (INFτ) and prostaglandins, as well as the expression of oxytocin receptors and certain genes activated by INFτ [18,24,43]. This physiological scenario can generate changes in the critical levels of certain hormones and growth factors that might compromise the correct progression of maternal recognition of pregnancy, conceptus survival, and embryo implantation [17,24,44]. In our study, the administration of two doses of hCG on days 7 and 14 did not show favorable results in some response variables. However, in other studies [11,16,30] using different hCG doses in small ruminants, higher levels of plasma progesterone were observed, in addition to better performance in other physiological parameters. Therefore, in future research, it would be interesting to correlate reproductive response variables, such as ovulation rate, percentage of ovulation, diameter of the corpus luteum, and luteal area with respect to plasma progesterone levels. Regarding the variables ovulatory rate, corpus luteum diameter, number of embryos, and embryo implantation rate, they did not differ among treatments. In this respect, it is important to consider that both hCG doses could have achieved the minimum required threshold in order to promote a similar reproductive response between treatments. Such results are in line with previous studies evaluating the use of hCG in seasonal anestrus goats, observing a similar effect of hCG on ovulatory rate [11,13]. The core idea, or central aim of this study was to evaluate the possible similar action of 100 or 300 IU of hCG upon some reproductive out-of-season outcomes in female goats under marginal production systems. A possible explanation of such lack of effect in the 100 IU-groups could be related to the extremely high sensitivity of the hypothalamic–pituitary axis to the negative retroaction of the gonadal E2 experimented by goats that were facing a deep anestrus. Most hormonal actions depend upon certain thresholds in order to provoke a defined response. So, a low hCG dose, a deep anestrous phase, added to a marginal production system may have conspired against better reproductive upshots. Another key issue is that the greater the size of the evaluated population, the largest the statistical robustness of the obtained results. Certainly, while non-larger enough replicate number tend to mask interesting results regarding the use of exogenous hormones to enhance out-of-season reproductive outcomes, the measurement of different metabolic and reproductive hormones, as well as diverse blood analytes should increase the possibility to better understand the diverse interactions occurring at physiological level while affecting the observed outcomes. Such issues must be clearly considered in future studies.

Embryo implantation is a very important and complex process which depends on an intricate while sophisticated crosstalk among the ovary, the uterus, and the conceptus; this complex process is fundamental since the highest rate of gestational losses occurs during

the embryo pre-implantation period [8]. The increase in the levels of both INFτ [23,45] and P4 [23,46] augments the likelihoods of a successful embryo implantation in ruminants. In our study, no differences occurred regarding the EIR between hCG doses, similar to that observed in other studies evaluating the use of hCG upon embryo implantation [22]; yet, the administration of hCG enhanced other reproductive variables with respect to the use of GnRH and the control group [47]. Interestingly, in our study, the largest luteal area was observed in the G300-14 group, agreeing with a previous study evaluating 300 IU of hCG 7d post-estrus in Toggenburg goats under an intensive system (21° South), observing a positive relationship between luteal area and hCG dose [48]. Such a physiological scenario also coincides with other studies using different hCG doses in small ruminants under different production systems (i.e., extensive vs. intensive), across different years/seasons, and diverse latitudes, concluding that the use of hCG was able to induce estrous activity [11,15,16,49,50].

Undoubtedly, the greater the size of the evaluated population, the largest the statistical robustness of the obtained results. Certainly, while non-larger, enough replicate numbers tend to mask interesting results regarding the use of exogenous hormones to enhance out-of-season reproductive outcomes, the measurement of different metabolic and reproductive hormones, as well as diverse blood analytes should increase the possibility to better understand the diverse interactions occurring at the physiological level while affecting the observed outcomes. Such issues must be carefully considered in future studies.

The maternal recognition of pregnancy is a process by which the conceptus indicates its presence to the mother in order to prolong the life of the corpus luteum, the P4 release while enhancing the pregnancy rate [17,24]. In order to ponder the success of the embryo implantation rate, with respect to the fertility and fecundity rate, two embryo implantation efficiency indices were constructed in our study: EEI1 and EEI2. The values generated for EEI1 favored the G300-14 group, while the observed EEI2 values favored the G300-7 and G300-14 groups, heightening the maternal recognition of pregnancy. This scenario suggests that the G300-14 had increased levels in either INFτ or in the receptors to the said molecule, at either the endometrial or ovarian level, enhancing the antiluteolytic mechanism destined to preserve the progestational state indispensable for the success of embryo implantation. In ruminants, the undeniably antiluteolytic signal is INFτ produced by trophoblast cells [24,42]. Taking into account both the time of application with respect to the FTAI and the half-life of hCG (i.e., about 40 h), in the G300-14 group, the embryo implantation rate could had been favored by enhancing the action of INFτ, which under normal conditions, it is mostly released on days 16 and 18 after the fertilization process. One of the main actions of INFτ is to prevent the pulsatile release of prostaglandin F2a (PGF2a) whose primary objective is to promote luteolysis or regression of the corpus luteum [17,51]. Therefore, the positive effects of exogenous hCG on goat embryonic development cannot be ruled out [52], and would explain the possible physiological scenario exerted by the G300-14 experimental group.

The precise site of action of hCG upon its positive effects regarding the embryo implantation success cannot be established without further studies. At the endometrial level, and considering a genomic approach, INFτ not only induces or activates various INF-stimulated genes (*ISGs*), but also positively regulates various genes closely related to the conceptus elongation process, its implantation, and the establishment of pregnancy [51,53]. Interestingly, the *IFNT* genes have only been described in ruminants within the genus Artiodactyla (i.e., cattle, sheep, and goats), emerging from an ancestral gene 36 million years ago, and over time, surely through rearrangements, and/or indel events that combined specific arrangements in such an ancestral gene (i.e., INF-ω) with a trophoblast-specifying promoter–enhancer domain [54,55]. While there is a low INFτ expression in the blastocyst stage, a massive upregulation occurs during the initial stages of the conceptus elongation; the INFτ expression vanishes with the attachment of the trophectoderm to the uterine endometrium. The main promoter element that controls expression is an ETS-2/AP-1 enhancer element [55]. Growth factors and cytokines released by the maternal endometrium

have been implicated in the control of *IFNT* gene transcription through the activation of ETS-2 [54]. This timely expression of *IFNT* is not only necessary to rescue the corpus luteum to secure the gestation process, but can also be an indicator of the suitability of the conceptus, thus acting as a critical factor dictating the continuation of gestation in goats and other ruminants. Moreover, *IFNT* acts through STST1/STA2-independent paths to uphold the activation of *ISGs*, supporting not only the P4 release by the small and large luteal cells, but also enhancing the transport of glucose and amino acids essential for the growth and development of the conceptus [18]. Much remains to be revealed; therefore, it is essential to endure with the assessment of novel, but innovative, reproductive and nutritional strategies that allow avoiding embryonic losses during the embryo implantation process, which, together with the free life period of the fertilized ovum, are critical to transit towards optimal reproductive efficiency outcomes of the female goat.

5. Conclusions

The response variables—conception rate, fertility rate, ovulation rate, corpus luteum diameter, embryo number, prolificacy rate, and embryo implantation rate—did not differ among experimental groups. Nonetheless, the 300 IU dose of hCG administered 14 d after the fixed-time artificial insemination protocol improved fecundity rate, corpus luteum area, as well as the embryonic efficiency index 1, while enhanced embryonic efficiency index 2 values were favored by both the G300-7 and G300 14. EEI1 and EEI2 were developed in order to ponder the success of the embryo implantation rate with respect to both the conception and fecundity rates; once such outcomes were weighed, the G300-14 group displayed the best embryonic implantation efficiency values. The present study contributes to attaining a better understanding, regarding the use of exogenous hormones that favor the reproductive performance of previously anestrus females. Consequently, such a reproductive strategy emerges as an interesting approach, not only to enhance the out-of-season reproductive responses, but also to boost one of the main rulers defining the global reproductive efficiency of a heard, namely, the embryo implantation rate. These results are of physiological importance and reproductive significance to the goat industry, and may embrace potential translational applications.

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ESTUDIO 2

ARTICULO 2: Exogenous hCG decreases early fetal losses and improves embryonic efficiency in rangeland goats subjected at a FTAI protocol during the reproductive transition season.

Exogenous hCG decreases early fetal losses and improves embryonic efficiency in rangeland goats subjected at a FTAI protocol during the reproductive transition season

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Abstract

The objective of this research work was to evaluate the possible effect of two doses of hCG (100 and 300 IU) administered at two different times (7 and 14 d) after fixed-time artificial insemination (FTAI) on some response variables related to early fetal loss and embryonic efficiency in goats during the reproductive transition period (June, 25° N). The experimental units were crossbred-multiparous goats (n = 48, Alpine, Saanen, Nubian x Criollo), with average body weight (45.3 ± 1.42 kg) and body condition score (1.96 ± 0.10), the study was conducted in the semiarid north of Mexico (25° N and 103° W). The goats (n=40) were subjected to an estrus induction protocol, subsequently the goats were inseminated, then the goats were randomly distributed into five experimental groups: 1). G100-7 (n = 8) 100 IU hCG, 7 d post-FTAI; 2). G100-14 (n = 8) 100 IU hCG, 14 days post-FTAI; 3). G300-7 (n = 8) 300 IU hCG, 7 d post-FTAI; 4). G300-14 (n = 8) 300 IU hCG, 14 days post-FTAI; and 5). CONT (n = 8) 0.5 mL of saline solution, 7 and 14 days post-FTAI. The response variables ovulation rate, diameter of the corpus luteum, number of embryos, fetal losses between d 30 and d 45 and from d 45 to parturition, prolificacy rate, weight of the kids at birth, there was no difference between treatments. However, the variables of corpus luteum area, embryonic implantation rate, embryonic efficiency index 1 and 2, conception rate, fertility rate, fecundity rate, fetal losses at d 30, d 45 and total favored G300- 14. Such a reproductive strategy of the use of hCG (300 IU) in the reproductive transition period is effective in reducing early

fetal losses, and improves the rate and embryonic efficiency, fundamental in the reproductive success of marginal goat production systems.

Key words: goats, reproduction, fetal losses, embryonic efficiency.

1. Introduction

Extensive goat production systems worldwide have been established in arid and semi-arid zones with low capacity from the vegetative point of view, for which the use is generally under grazing conditions (Escareño et al., 2013). Reproductive performance in these production systems is a fundamental component in milk and kid production (Mellado and Meza-Herrera, 2002; Mellado, 2008). In Mexico, in the last decade approximately 5 million goats have been found in extensive systems in arid and semi-arid zones (Escareño et al., 2013; Navarrete-Molina et al., 2020) in the Comarca Lagunera, north of in Mexico, the goats inventory exceeds 390 thousand (SIAP, 2021). The goats of this region are classified as seasonal polyestrous, because there is seasonality in the production of milk, kid and derivatives (Álvarez et al., 2001; Meza Herrera et al., 2019), there is a period of deep anestrus from March to May and a breading season that begins in August and end in February, with a reproductive transition stage from June to July (Contreras-Villarreal et al., 2015; Alvarado-Espino et al., 2016). Hormonal strategies such as the use of hCG have been used to counteract the effect of deep seasonal anoestrus and the reproductive transition in the region (Alvarado-Espino et al., 2019a; Bustamante-Andrade et al., 2021). The administration of hCG in different doses (example: 600, 500, 300, 100 and 50 IU) directly stimulate the dominant ovarian follicles in advanced stages of development, triggering ovulation, formation of the corpus luteum, increase in the concentration of progesterone and in embryonic efficiency in goats in anestrus and in reproductive transition, improving embryonic implantation (Coleson et al., 2015; Dias et al., 2018; Alvarado-Espino et al., 2019b). Most pregnancy losses in goats occur during the preimplantation stage, which is therefore the critical period that determines reproductive success (Pokarel et al., 2020). Therefore, failure in early pregnancies in this species has as its main cause the deficiency of luteal function and as a consequence early embryonic mortality (Diskin and Morris 2008; Khan et al., 2009; Bazer et al., 2020) in addition,

asynchrony between the embryo and the uterus causes insufficient development, which is manifested by a reduction in the signaling of the embryo for the establishment of gestation and a decrease in the luteotrophic effect (Spencer et al., 2004; Fernandez et al., 2019) concluding with the physiological process of embryo implantation (Pohler et al., 2015; Peter et al., 2017). In a recent study in the Comarca Lagunera, a high dose of hCG (300 IU) 14 days after Fixed Time Artificial Insemination (FTAI) is effective in luteogenesis and embryonic efficiency, in goats in seasonal anoestrus managed under a system of extensive production (Bustamante -Andrade et al., 2021). Therefore, in this study we hypothesize that the administration of 300 IU of hCG after FTAI decreases early fetal loss, as well as increases embryo implantation rate and litter size in goats during the reproductive transition period managed under extensive rangeland conditions.

2. Materials and Methods

2.1. General

All experimental procedures, methods, and handling of the experimental units used in this study complied with international (FASS, 2010) and national (NAM, 2010) guidelines for the ethical use, care, and welfare of research animals. In addition, institutional approval was obtained for its realization (UAAAN-UL-18-3059).

2.2 Description of location and environmental conditions

The study was carried out in the arid North of Mexico, in the Comarca Lagunera region (25° N, 103° W, altitude= 1111 m), this region has a dry climate, with an average annual temperature of 21 ºC (37 ºC from May to August in summer and 0 ºC from November to February in winter), rainfall occurs from June to September with an annual average of 266 mm (INEGI, 2015).In the Comarca Lagunera, goat production predominates under the sedentary extensive rangeland system, the goats feed on the native vegetation and agricultural waste (INIFAP, 2010).

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99 *2.3 Animals and their management*

100 The goats were milked manually once a day (07:00 a.m.) and then taken out to graze
101 (from 10:00 a.m. to 06:00 p.m.), 45 d before starting the experimental phase, the
102 herd was dewormed subcutaneously (Ivermectin 1%, Baymec, Bayer, Mexico City,
103 Mexico), vitaminized with vitamins A (500,000 IU), D3 (75,000), E (50 mg) and
104 Vigantol (ADE + Selenium 250 mL, Zapopan Jalisco Mexico). One month before the
105 start of the study; water, shades and mineral salts (17 % P, 3 % Mg, 5 % Ca and 75
106 % NaCl) were available and freely accessible.

107 2.3.1. Goat females

108 *Selection of goats*. From a commercial herd of multiracial-crossbred goats (n = 155;
109 Alpina, Saanen, Nubian x Criollo), 48 multiparous goats, with 45.3 ± 1.42 kg of body
110 weight, 1.96 ± 0.10 units body condition score and 2 -4 lactations, were selected and
111 identified with earrings for better management during the experimental period.

112 *Estrus induction protocol*. Ovarian activity was determined in June by transrectal
113 ultrasonography (5.3-10 MHz color Doppler equipment, Chison ECO-5, with 12-inch
114 probe). Each goat underwent ultrasound on days 14 and 7 before the application of
115 hCG, in order to confirm the presence of corpora lutea in both ovaries. Goats (13/48=
116 27.08%) that resulted whit functional corpora lutea were given 1 mL of an analog of
117 prostaglandin F_2 alpha (D -Cloprostenol® Sanfer, i.m., Ciudad de México, CDMX)
118 with the aim of inducing lysis of the corpus luteum. Once the absence of corpora
119 lutea was confirmed, the goats were subjected to an estrus induction protocol. On
120 day –1 d, all females received 20 mg of P4 (Progesvit®, Brovel, i.m., Irapuato,
121 Guanajuato, Mexico) to avoid the presence of short cycles. On day zero (0 d), the
122 goats received 200 IU of hCG (Chorulon®, Intervet, Mexico City, Mexico) i.m., in
123 order to stimulate the formation of the antrum in the ovarian follicles in advanced
124 stages of development, and promote, in turn, ovulation. The next day in the morning,
125 for the detection of estrus, three sexually active adult male goats provided with

aprons were used to avoid copulation with the female. Of the total number of goats (n=48) exposed to the induction protocol, 40 responded showing estrus.

2.3.3. Male goats: extraction and evaluation of semen quality

The male goats (n = 3; Grenadine breed; 2.5 years old), used for semen collection, were subjected to treatment with the application of 50 mg of testosterone (Testosterone-50, androgenic steroid, i.m., Lab. Brovel, Mexico City, Mexico) every third day for three weeks before the start of the study period, to improve spermatogenesis (Luna-Orozco et al., 2012). At the end of June, semen was extracted using an artificial vagina (Walmur-Veterinaria Montevideo, Uruguay); the ejaculate was evaluated and only ejaculates with volume ≥0.5 mL, sperm concentration ≥2500 X 106 cells/mL and sperm mass motility ≥3 (scale 0-5) and progressive ≥70% were used. In order to avoid any effect on the embryo implantation rate, the collected semen samples were mixed and subsequently diluted using a commercial diluent and following the instructions provided by the laboratory (OptidylTM, Cryo-Vet, León Guanajuato, Mexico).

2.4. Fixed Time Artificial Insemination (FTAI)

All goats (n = 40) were exposed to the IATF protocol 48 h after the application of hCG. The insemination procedure was carried out using a vaginoscope (Walmur-Veterinary, Montevideo, Uruguay) equipped with a light source. The semen was deposited in the pericervical area and two services were given in the morning (9:00 a.m.) and in the afternoon (7:00 p.m.).

2.5. Conformation of the experimental groups

Once all the goats were inseminated (n = 40), they were distributed in five experimental treatments considering two doses of hCG (100 and 300 IU, Chorulon®, Intervet, Mexico City, Mexico) and two application times (7 and 14 d post-FTAI), plus a Control group. Therefore, the experimental groups were formed as follows: 1). G100-7 (n = 8), 100 IU of hCG, 7 days after FTAI; two). G100-14 (n = 8), 100 IU of hCG, 14 days after FTAI; 3). G300-7 (n = 8), 300 IU of hCG, 7 days after FTAI; 4).

G300-14 (n = 8), 300 IU of hCG 14 days after FTAI, and 5). CONT (n = 8), 0.5 mL saline 7 and 14 days after FTAI.

2.6. Response variables

2.6.1. Body weight, body condition score and estrus induction.

Body weight (BW) and body condition score (BCS) were recorded at the beginning of the experimental period. To determine the PC, a digital scale with a capacity of 250 kg and a precision of 50 g (Torrey 110v / 220v Jalisco, Mexico) was obtained. Additionally, CC was determined by an experienced technician as previously described by Walkden-Brown et al. (1997), considering a scale from 1 (very thin) to 4 (very fat). Once the absence of corpora lutea was granted, the goats were subjected to the oestrus induction protocol mentioned above.

2.6.2. Ovulation rate, corpus luteum diameter, luteal area, embryo implantation rate, and embryo efficiency indices.

The percentage of females that ovulated was determined on days 0 and 10 after the estrus induction protocol and was calculated based on the observation of corpora lutea (CL) by transrectal ultrasound evaluation (5.3-10 MHz of a Chison ECO-5 color Doppler equipment, with 12-inch test). The ovulation rate (OVR) was calculated by observing CL through ultrasound on day 10 after FTAI, in addition, the luteal area (CLA) was determined by measuring the diameter of the corpus luteum (CLD). At day 30 post-IATF, the embryo implantation rate (EIR) was determined by the same route. In addition, two indices were developed in order to weigh the success of the embryo implantation rate with respect to the conception rate as the fecundity rate, the Embryo Efficiency Index 1 [IEE1= (embryo implantation rate)(conception rate /100)] , and embryonic efficiency index 2 [IEE2= (embryonic implantation rate)(fecundity rate/100)].

2.6.3. Rates of conception, fertility, fecundity and prolificacy.

The conception rate (CR) was determined on day 45 post-IATF, considering the number of pregnant goats / number of inseminated goats; for the fertility rate (TFR), the number of pregnant females that gave birth was taken into account; for the fecundity rate (FCR), the number of fetuses per inseminated goat was considered; and the prolificacy rate (PR), was determined at parturition considering the number of offspring born per pregnant goat.

2.6.4. Early fetal losses, litter size and birth weight.

Fetal loss percentages at day 30, 45 post-FTAI and until delivery were determined from transrectal ultrasonography. The birth weight of the kids was recorded immediately after the mother finished cleaning it, in order not to disturb the establishment of the mother-calf bond. For the birth weight, a scale was used with a capacity of 40 kg and a precision of 5 g (Torrey LPCR 40 USB 40 kg/ 5g port USB A/B. Jalisco Mexico).

Therefore, the possible effect of these five experimental treatments on early fetal losses, embryonic efficiency, litter size and other reproductive variables, is shown in Figure 1.

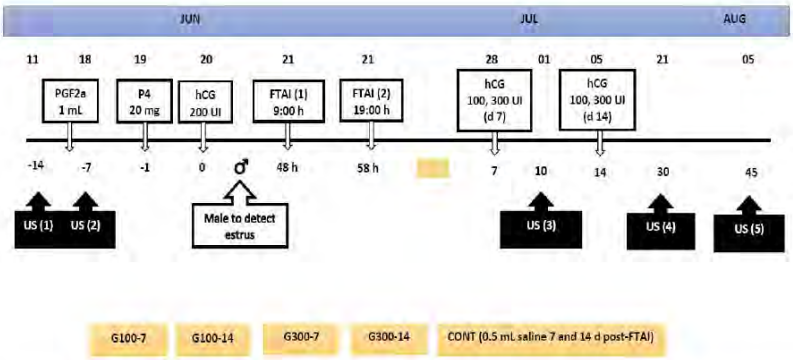

Figure 1. Schematic representation of the experimental protocol. The application of hCG (100 or 300 IU) was on day 7 or 14 post-IATF. Transrectal ultrasonography was performed

to determine the ovulatory status (US1 and US2), ovulatory rate (US3), embryo implantation rate (US4) and conception rate (US5), in goats kept under an extensive system and in reproductive transition (June, 25°N).

2.7. Statistical analysis

A first linear model was developed to assess the potential relationship of hCG dose (ie, 100 vs. 300 IU) and day of administration (ie, 7 vs. 14 days after FTAI) with respect to body weight (BW, kg), diameter of the corpus luteum (CLD, mm), area of the corpus luteum (CLA, mm^2). Regarding percentage and count variables: body condition score (BCS, units), estrus induction (EI,%), conception rate (CR,%), fertility rate (FR,%) prolificacy rate (PR,%), fecundity rate (FCR ,%), ovulation rate (OVR, units), number of embryos (EN, units), Embryo Implantation Rate (EIR,%), Embryo Efficiency Index-1 (IEE -1,%) and embryonic efficiency index-2 (IEE -2,%), and fetal losses at d 30, d 45, d 30-45, d 45 at delivery and total (%) were transformed log^{10} before performing the ANOVA to overcome skewness, as the data does not fit the normal distribution . Least squares means and standard errors were computed for each experimental treatment, multiple comparisons of means were performed using the Fisher's LSD – LSMEANS option of the SAS PROC GLM. Since all experimental treatments were evaluated individually, each goat within the experimental group was defined as the experimental unit. Differences in treatment are accepted if $p < 0.05$. All analyzes are calculated using SAS procedures (SAS Inst. Inc. Version 9.4, Cary, North Carolina, United States).

3. Results

3.1. Body weight, body condition score and estrus induction.

These variables did not show statistical differences ($p > 0.05$) between experimental groups, in general, the average value of body weight was 45.34 ± 1.42 kg, body condition score was 1.96 ± 0.10 units, and estrus induction was 83.5% (Table 1).

233 Table 1. Least squares means ± standard error of body weight (BW), body condition score
234 (BCS) and estrus induction (EI) of multibreed and multiparous goats (n = 40, Alpine, Saanen,
235 Nubian x Criollo) managed under extensive conditions in northern Mexico and treated with
236 100, 300 IU of hCG or saline solution at 7 or 14 days post Fixed Time Artificial Insemination,
237 during the reproductive transition season (June, 25°N).

Variables	G100-7 (n=8)	G100-14 (n=8)	G300-7 (n=8)	G300-14 (n=8)	CONT (n=8)	*p Value*
BW (kg)	45.6 ± 1.84	44.8 ± 1.14	45.2 ± 1.06	45.7 ± 1.60	45.4 ± 1.49	0.90
BCS (units)	2.0 ± 0.09	1.9 ± 0.07	2.0 ± 0.13	2.0 ± 0.08	1.9 ± 0.13	0.56
EI (n, %)	8/10 (80.0)	8/9 (88.8)	8/10 (80.0)	8/10 (80.0)	8/9 (88.8)	0.90

238 [1] No differences (p > 0.05) for any variable occurred among experimental groups.

239

240 *3.2 Ovulation rate, corpus luteum diameter, luteal area, embryo implantation rate,*
241 *and embryo efficiency indices.*

242 There was no statistical difference (p>0.05) between treatments for the response
243 variables ovulatory rate (1.11 ± 0.30 units), diameter of the corpus luteum (10.6 ±
244 0.71 mm) and number of embryos (1.72 ± 0.25). In contrast, the higher embryo
245 implantation rate and the larger corpus luteum area (p<0.05) favored the
246 experimental group G300-14 (Table 2). However, although there were no statistical
247 differences (p>0.05) between groups G100-7, G100-14, G300-7 and CONT for both
248 efficiency indices (EEI1 and EEI2), the higher values for indices favored (p<0.05) to
249 G300-14 with respect to the rest of the treatments.

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Table 2. Least squares means ± standard error of ovulation rate (OVR), corpus luteum diameter (CLD), corpus luteum area (CLA), number of embryos (EN), embryo implantation rate (EIR), embryonic efficiency index-1 (IEE-1) and embryonic efficiency index-2 (IEE-2), of multiracial and multiparous goats (n = 40, Alpine, Saanen, Nubian x Criollo) managed under extensive conditions in northern Mexico and treated with 100, 300 IU of hCG or saline solution 7 or 14 days after Fixed Time Artificial Insemination, during the reproductive transition season (June, 25°N).

Variables	G100-7 (n=8)	G100-14 (n=8)	G300-7 (n=8)	G300-14 (n=8)	CONT (n=8)	*p Value*
OVR (n)	0.87 ± 0.33	1.1 ± 0.48	1.4 ± 0.17	1.5 ± 0.33	0.68 ± 0.17	0.46
CLD (mm)	10.6 ± 0.42	9.9 ± 0.45	10.9 ± 0.96	12.9 ± 0.72	8.68 ± 0.98	0.23
CLA $(mm)^2$	87.35 ± 0.18^b	97.58 ± 0.32^b	100.11 ± 0.45^b	135.66 ± 0.25^a	95.02 ± 0.36^b	0.04
EN (n)	1.60 ± 0.18	1.80 ± 0.42	1.80 ± 0.20	2.0 ± 0.26	1.40 ± 0.20	0.59
EIR (n, %)	5/8 (62.5)^b	5/8 (62.5)^b	6/8 (75.0)^b	8/8 (100.0)^a	3/8 (37.5)^b	0.03
EEI^1 (%)	23.43 ± 0.25^b	31.25 ± 0.21^b	46.87 ± 0.21^b	87.50 ± 0.20^a	14.06 ± 0.24^b	0.02
EEI^2 (%)	31.25 ± 0.33^b	23.43 ± 0.21^b	37.5 ± 0.29^b	87.50 ± 0.33^a	9.37 ± 0.29^b	0.03

Different letters between columns show difference ($p > 0.05$). Data are presented as mean standard error of the mean. Embryo Efficiency $Index^1$ = [implantation rate] [conception rate/100]. Embryo Efficiency $Index^2$ = [implantation rate] [fecundity rate/100].

3.3 Rates of conception, fertility, prolificacy and fecundity.

As seen in Table 3, the prolificacy rate was not statistically different ($p>0.05$) between the treatments, showing the following average (1.5 ± 0.29 pups), that is, there was no effect of the two doses of hCG (100 vs. 300 IU) at different times (7 vs 14 d) after FTAI with respect to GCONTROL. However, the variables of conception rate, fertility rate at delivery and fecundity rate showed significant statistical difference ($p=0.04$; $p=0.05$; $p=0.03$) favoring the experimental group G300-14.

279 Table 3. Least squares means ± standard error of conception rate (CR), fertility rate (FR),
280 prolificacy rate (PR) and fecundity rate (FCR) of multibreed and multiparous goats (n = 40,
281 Alpina, Saanen, Nubian x Criollo).) managed under extensive conditions in the north of
282 Mexico and treated with 100, 300 IU of hCG or saline solution at 7 or 14 days after Fixed
283 Time Artificial Insemination, during the reproductive transition season (June, 25 °N).

VARIABLES	G100-7 (N=8)	G100-14 (N=8)	G300-7 (N=8)	G300-14 (N=8)	CONT (N=8)	*P VALUE*
CR (N, %)	3/8 $(37.5)^{B}$± 0.27	4/8 $(50.0)^{B}$ ± 0.14	5/8 $(62.5)^{B}$± 0.2	8/8 $(100)^{A}$ ±0.14	3/8 $(37.5)^{B}$ ± 0.1	0.04
FR (N, %)	3/8 $(37.5)^{B}$ ±0.09	3/8 $(37.5)^{B}$ ± 0.14	4/8 $(50.0)^{B}$ ± 0.1	7/8 $(87.5)^{A}$ ±0.25	2/8 $(25.0)^{B}$ ± 0.13	0.05
PR (N)	1.3 ± 0.23	1.5 ± 0.33	1.5 ± 0.33	2.0 ± 0.29	1.2 ± 0.29	0.25
FCR (N, %)	4/8 $(50.0)^{B}$ ± 0.2	3/8 $(37.5)^{B}$ ± 0.11	4/8 $(50.0)^{B}$ ± 0.1	9/8 $(112.5)^{A}$±0.2	2/8 $(25.0)^{B}$ ± 0.06	0.03

284 ^{a,b} Response variables with different superscripts within lines, differ (p > 0.05).

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286 *3.4. Early fetal losses, litter size and birth weight.*

287 As shown in Table 4, there were no differences (p>0.05) for the variables of fetal
288 losses between d 30 and d 45, as well as fetal losses from d 45 to delivery. In
289 contrast, the lower percentage of fetal losses at d 30 to d 45 post FTAI and total
290 favored G300-14 (p<0.05), which did not show fetal losses in these two periods, only
291 one after d 45. Regarding the weight of the pups at birth, there was no statistical
292 difference (p > 0.05) between the experimental groups and the CONT regardless of
293 the type of delivery, however, in the G300-14 (9 kids) it was the one that presented
294 the greatest number of offspring, in single births, twins and the only triple birth: the
295 rest of the groups had fewer kids G100-7 (4 kids), G100-14 (3 kids), G300-7 (4 kids)
296 and CONT (2 kids).

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Table 4. Least squares means ± standard error of fetal losses at d 30, d 45, between d 30 and d 45, from 45 to delivery; and birth weight of kids from multiracial and multiparous goats (n = 40, Alpina, Saanen, Nubia x Criollo) managed under extensive conditions in northern Mexico and treated with 100, 300 IU of hCG or saline at 7 or 14 days post Fixed Time Artificial Insemination, during the reproductive transition season (June, 25°N).

Variables	G100-7 (n= 8)	G100-14 (n= 8)	G300-7 (n=8)	G300-14 (n=8)	CONT (n=8)	*p Value*
Fetal losses at d 30 post FTAI (%)	3/8 (37.5±0.12)^{b}	3/8 (37.5±0.25)^{b}	2/8 (25.0±0.19)^{b}	0/8 (0.0)^{a}	5/8 (62.5±0.13)^{b}	0.04
Fetal losses at d 45 post FTAI (%)	5/8 (62.5±0.1)^{b}	4/8 (50 ± 0.13)^{b}	3/8 (37.5±0.15)^{b}	0/8 (0.0)^{a}	5/8 (62.5±0.18)^{b}	0.03
Fetal losses between days 30 and d 45 post FTAI (%)	2/8 (25 ± 0.12)	1/8 (12.5±0.23)	1/8 (12.5 ±0.2)	0/8 (0.0)	0/8 (0.0)	0.53
Fetal losses between d 45 post FTAI and the birth (%)	0/8 (0.0)	1/8 (12.5 ± 0.25)	1/8 (12.5±0.14)	1/8 (12.5±0.1)	1/8 (12.5 ±0.25)	0.76
Total fetal loss (%)	5/8 (62.5 ±0.2)^{b}	5/8 (62.5 ±0.2)^{b}	4/8 (50 ± 0.10)^{b}	1/8 (12.5±0.2)^{a}	6/8 (75 ± 0.1)^{b}	0.02
Birth weight of kids (kg)						
Single (n= 11)	3.8±0.51 (2)	3.5±0.32(1)	3.7±0.42(2)	3.6±0.5 (4)	3.5±0.6 (2)	0.45
Twin (n= 8)	2.6±0.15(2)	2.4±0.23(2)	2.8±0.56(2)	2.9±0.63(2)	nd* (0)	0.65
Triple (n=3)	nd* (0)	nd* (0)	nd* (0)	2.2±0.36(3)	nd* (0)	-----
Totals (n=22)	(4)	(3)	(4)	(9)	(2)	0.10

$^{a, b}$: Different letters in the same row indicate P < 0.05 (Statistical significance). *nd: not data.

4. Discussion

The results obtained in this study supported our hypotheses, which establishes that a high dose of hCG (300 IU) is effective in reducing early fetal losses, in addition, it is effective in embryonic efficiency and in the size of the litter In this sense, the highest values of the response variables: conception rate (CR), fertility rate (FR), fecundity rate (FCR), corpus luteum area (CLA), embryo implantation rate (EIR) , embryonic efficiency index 1 and 2 (IEE1, EEI2); they were higher in G300-14 with the administration of the high dose of hCG (300 IU) two weeks (14 d) after the

317 application of the protocol. The findings found in this study coincide with what was
318 previously reported by (Bustamante-Andrade et al., 2021) who evaluated the effect
319 of hCG doses post FTAI in goats in an extensive sedentary system in deep seasonal
320 anoestrus (April, 25° LN) in Comarca Lagunera, and found better results with the
321 high dose of hCG on luteogenesis, embryonic efficiency; surely due to the dual effect
322 of this exogenous hormone that coincides with the function of gonadotropins: LH and
323 FSH and consequently the better development of ovarian structures, (Rostami et al.,
324 2017; Días et al., 2018) promoting the process of embryonic establishment in a
325 tripartite synchrony between the embryo, the maternal recognition of gestation and
326 the receptivity of the uterus, thus reducing fetal losses. (Bai et al., 2012). As it
327 happened in this study where we found a statistical difference in the lowest values
328 of fetal losses at d 30, at d 45 and total, favoring the G-300 14 with respect to the
329 rest of the groups. In effect, this research helps to reduce the negative effect of early
330 fetal loss and thus increase litter size through the use of hormonal protocols in
331 female goats in the reproductive transition stage, promoting better physiological and
332 endocrine conditions in the critical period of gestation.

333 At the beginning of the experimental period in our study, 83.33% of the goats (40/48)
334 responded favorably to the estrus induction protocol. In various studies in the
335 Comarca Lagunera, similar induction protocols have been used, with 20 mg of P4
336 plus hCG in different doses, for example that reported by (Alvarado-Espino et al.,
337 2016; Alvarado-Espino et al., 2019a) who used different doses of hCG (0, 50, 100
338 and 300 IU) in goats during the early reproductive transition period (June) and
339 reported an effect close to 90% of females in estrus. In reference to the variables of
340 body weight (BW) and body condition score (BCS) they were not different between
341 the experimental groups and the CONT because the goats were selected based on
342 the homogeneity of these variables for the conformation of the groups.

343 In reference to the response variables of ovulation rate (OVR), diameter of the
344 corpus luteum (CLD) and number of embryos (EN), they were not different between
345 the treatments, most likely due to the effect of the reproductive transition period, that
346 is, the goats required less energy for these physiological processes, in this sense

these results are consistent with those found in local goats of the Comarca Lagunera in the period of early reproductive transition (Alvarado-Espino et al., 2016; Alvarado-Espino et al. al ., 2019b) for the same variables, however the embryo implantation rate was different between the treatments, favoring the group that received the high dose of hCG, G300-14, promoting the highest embryonic efficiency against the rest of the treatments, these results coincides with what was reported in different studies in small ruminants where they found higher embryo implantation rates (Spencer et al., 2007; Brooks et al., 2016; Rahem, 2018). A possible explanation for such a lack of effect in the 100 IU groups could be related to the extremely high sensitivity of the hypothalamus–pituitary axis to negative gonadal E2 feedback experienced by goats facing a reproductive transition period i.e. coming from a seasonal anoestrus, most hormonal actions depend on certain thresholds to elicit a definite response. It is interesting to mention that in our study the largest luteal area (CLA) favored the G300-14 group, coinciding with an investigation in which they evaluated 300 IU of hCG 7d post estrus in Toggenburg goats (Côrtes et al., 2020) under intensive system (21° South), observing a positive relationship between the luteal area and the high dose of hCG, likewise (Bustamante-Andrade et al., 2021) reports similar findings in local goats from the Comarca Lagunera in seasonal anoestrus treated with different doses of hCG, in a marginal production system. To weigh the success of the embryo implantation rate, with respect to the rate of conception and fertility, in our study embryonic efficiency indices were established: IEE1 and IEE2. The values generated by both indices favored the G300-14 group, increasing the process of maternal recognition of pregnancy, for which we establish that a high dose of hCG at this physiological stage is necessary for greater synthesis and function of IFN (Raheem, 2015; Bazer et al., 2020; Al-Samawi et al., 2021); for example the antagonistic effect with respect to prostaglandin F2a to prevent regression of the corpus luteum (Pokharel et al., 2020).

Regarding the response variable prolificacy rate (PR) did not differ between the experimental groups of this study, this information coincides with that reported by (Rodríguez Martínez et al., 2017) who found a prolificacy rate of (1.55 ± 0.02) in a study where hCG was used in goats from the Comarca Lagunera, however the

highest values for the variables of conception rate (CR), fertility rate (FR) and fecundity rate (FCR) favored the G300-14 group is say the proportion of the number of fetuses with respect to the number of inseminated females was influenced by the effect of the high dose of hCG at 14 d post IATF, these results agree with (Fonseca et al., 2017; Fernandez et al., 2019) who carried out a study where the conception rate was similar using hCG, in addition to other exogenous hormones at different doses in goats in Brazil; In another study carried out on sheep of the Merino breed, GnRH (4 µg i.m.) and hCG (300 IU i.m.) were administered on day 4 post-FTAI in northern Patagonia and I found the best results for fertility and fecundity rates. The success in the gestation of goats as in other domestic ruminants is conditioned by the adequate functionality of the corpus luteum for the release of progesterone (Spencer et al., 2007; Raheem, 2018; Pokharel et al., 2020); This hormone is closely related to the formation and functioning of interferon-tau (INFt) for the process of early recognition of pregnancy, together with prostaglandin and oxytocin (Spencer et al., 2004; Chen et al., 2013; Bazer et al. , 2020; Pokharel et al., 2020; Al-Samawi et al., 2021).

Regarding the variables related to early fetal loss, in our research we found that the best results favored the G300-14 group, which did not present early fetal loss during the first 30 and 45 days after FTAI and presented only one fetal loss. Unlike the rest of the treatments, these results are in line with what was reported by (Fernandez et al., 2019) who carried out a study in sheep of the Merino breed administering GnRH (4 µg i.m.) and hCG (300 IU i.m.) at day 4 post IATF in northern Patagonia and found a statistical difference in the lower number of early fetal losses (d 33 post IATF) in the group of sheep in which hCG was used. Early fetal losses in small ruminants are estimated to range from 8% - 30% before day 30 of gestation (Rickard et al., 2017; Smith et al., 2018). These early losses coincide with the expansion of the concept and the placentation. In recent studies, it was shown that treatment with hCG, used in the early luteal phase in sheep, showed a positive effect in reducing early fetal loss, probably related to the formation of accessory corpora lutea; in this sense (Bartolomé et al., 2012; García-Pintos and Menchaca 2017) establish that the luteotrophic action of hCG produces an increase in the concentration of

progesterone necessary for an adequate intrauterine environment, to improve embryonic survival and therefore reduce fetal losses in ruminants. Regarding the weight of the pups at birth, there was no statistical difference between the experimental groups and the CONT regardless of the type of delivery, however in the G300-14 it was the one that presented the largest number of pups, in simple births, twins and the only triple birth was in this group. That is, litter size was larger for this experimental group. In this sense, research carried out in goat production systems in marginal conditions has reported an incidence of up to 70% of fetal losses in mestizo goats in Mexico, affecting the global prolificacy of the herds (Mellado, 2008). In another study carried out by (Fernandez et al., 2019) they report a larger litter size in sheep of the Merino breed under extensive grazing conditions, when hCG was applied (n=38 kids) with respect to GnRH (n= 28 kids).) in the breeding season in Argentina.

The proposal that we establish with the use of high-dose hCG in goats in the reproductive transition season represents an alternative solution to the problem of early fetal loss, reduced embryonic efficiency and litter size, which goat farmers face. in extensive production systems, due to the fact that at this time of year the goats require less metabolic demand to restart the reproductive cycle, which translates into substantial economic losses, for which we prioritize the use of reproductive biotechnology through the use of IATF, The use of hCG in extensive goat production systems to increase the reproductive efficiency of herds, through genetic improvement; however, it is a priority to continue scientific research in this species and under this production system because of what it represents for the world population.

5. Conclusions

Our results from this second confirm that a high dose of hCG (300 IU) two weeks (14 d) after the Fixed Time Artificial Insemination study is necessary to reduce early fetal losses in goats in the reproductive transition period, and by inherent consequence a greater embryonic efficiency, coupled with the increase in the size of the litter. Taking into account that the best results in the response variables:

fecundity rate (FCR), conception (CR), corpus luteum area (CLA), embryonic efficiency indexes 1 and 2 (IEE1 and IEE2), in addition to the lowest fetal losses at 30, 45 d post insemination and totals favored the experimental group G300-14. With the completion of this study, one more alternative is ratified for the increase in productivity in goat production systems through the use of the hormone hCG as a reproductive strategy to reduce embryonic mortality during the critical period of gestation, in the reproductive transition season due to the lower energy demand to restart the reproductive cycles in this specie.

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CONCLUSIONES GENERALES

ESTUDIO 1

Nuestros resultados confirman que las variables de respuesta: tasa de concepción, tasa de fertilidad, tasa de ovulación, número de embriones, tasa de prolificidad y tasa de implantación de embriones no difirieron entre grupos experimentales. No obstante, la dosis de 300 UI de hCG administrada 14 d después el protocolo de inseminación artificial a tiempo fijo mejoró la tasa de fecundidad, el área del cuerpo lúteo, así como el índice de eficiencia embrionaria 1. Los valores del índice 2 se vieron favorecidos tanto por el G300-7 como por el G300 14. Los indicies de eficiencia embrionaria fueron desarrollados para ponderar el éxito de la tasa de implantación embrionaria con respecto a las tasas de concepción como de fecundidad; el G300-14 grupo mostró los mejores valores de eficiencia de implantación embrionaria. El estudio presente contribuye a lograr una mejor comprensión respecto al uso de hormonas exógenas que favorecen el comportamiento reproductivo de hembras previamente en anestro. Como consecuencia, tal estrategia de control de la reproducción surge como un enfoque interesante, no sólo para mejorar el desempeño de los caprinos fuera de la estación reproductiva, sino también para impulsar uno de los principales factores que determinan su eficiencia reproductiva global, es decir, la tasa de implantación embrionaria. Estos resultados son de fundamental importancia para la eficacia de los sistemas de producción caprina.

ESTUDIO 2

Nuestros resultados de este segundo confirman que una dosis alta de hCG (300 UI) dos semanas (14 d) después del estudio de Inseminación Artificial a Tiempo Fijo es necesaria para reducir las pérdidas fetales tempranas en cabras en el período de transición reproductiva, y por consecuencia inherente una mayor eficiencia embrionaria, aunado al aumento del tamaño de la camada. Teniendo en cuenta que los mejores resultados en las variables de respuesta: tasa de fecundidad (FCR), concepción (CR), área de cuerpo lúteo (CLA), índices de eficiencia embrionaria 1 y 2 (IEE1 e IEE2), además de las menores pérdidas fetales al 30, 45 d post inseminación y los totales favorecieron al grupo experimental G300-14. Con la realización de este estudio, se ratifica una alternativa más para el aumento de la productividad en los sistemas de producción caprina mediante el uso de la hormona hCG como estrategia reproductiva para reducir la mortalidad embrionaria durante el período crítico de la gestación, en la época de transición reproductiva por la menor demanda energética para reiniciar los ciclos reproductivos en esta especie.