UNIVERSIDAD AUTÓNOMA AGRARIA ANTONIO NARRO

SUBDIRECCIÓN DE POSTGRADO

EFECTO DEL RANGO SOCIAL Y DE LA DOSIS DE eCG SOBRE LA INDUCCIÓN DE LA ACTIVIDAD REPRODUCTIVA EN CABRAS ANÉSTRICAS

Tesis

Que presenta SANTIAGO ZÚÑIGA GARCÍA

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

Torreón, Coahuila Julio 2020

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Torreón, Coahuila

Julio 2020

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Elaborada por SANTIAGO ZÚÑIGA GARCÍA 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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Julio 2020

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Al **Ser Supremo**, que siempre ha estado conmigo. Gracias por tu sabiduría.

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A mis hijas **Victoria y Valentina**, son el motor de mi existencia. Las amo con todo mi corazón.

CARTAS DE ACEPTACIÓN

Aceptación de artículo 1

[Animals] Manuscript ID: animals-839851 - Accepted for Publication

lun., 29 de junio de 2020 12:28 p. m.

Dear Dr. Meza-Herrera,

We are pleased to inform you that the following paper has been officially
accepted for publication:

Manuscript ID: animals-839851
Type of manuscript: Article
Title: Effect of social rank upon estrus induction and some reproductive
outcomes in anestrous goats treated with progesterone + eCG.
Authors: Santiago Zuñiga-Garcia *, Cesar Meza-Herrera, Adela
Mendoza-Cortina, Julio Otal-Salaverri, Carlos Perez-Marin, Noé M.
Lopez-Flores, Evaristo Carrillo, M. G. Calderon-Leyva, U.N. Gutierrez-Guzman,
Francisco G. Veliz-Deras
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Submitted to section: Animal System and Management,

Aceptación de artículo 2

AGRICULTURAL RESEARCH COMMUNICATION CENTRE

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Reference ID. ARCC/B-1251 **Date : 29-06-2020**

Francisco Gerardo Véliz Deraz,
Departamento de Ciencias Veterinarias,
Universidad Autónoma Agraria Antonio Narro,
Torreón, Coahuila, México.

Acceptance of article

Dear Dr. Gerardo,

We are pleased to inform you that your article has been accepted for publication in **Indian Journal of Animal Research.** Your submission is a well-thought out piece of writing and follows many of journal guidelines. The editors agreed that your submission showed great writing skills.

Article Title : Evaluation of different eCG doses + progesterone to induce reproductive activity during the transitional reproductive season in anestrous Creole goats

Author(s) : S. Zúñiga-García, G. Calderón-Leyva, J. Otal-Salaverri, S. Moreno-Avalos, F. Arellano-Rodríguez, F.G. Véliz-Deras

Congratulations to you once again on your article acceptance in ARCC Journals, and we look forward to receiving more of your good submissions.

With Best Wishes and Seasonal Greetings,

Sincerely,

(R.D. Goel)
Managing Editor

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RESUMEN

Evaluamos el efecto del rango social [R] (bajo-LSR, medio-MSR o alto-HSR) en cabras anéstricas, las cuales fueron sometidas a un protocolo de inducción del estro (EIP) basado en progesterona + eCG [D] (100 o 350 UI). Se utilizaron cabras (n=70) de un sistema intensivo. Se evaluó el efecto del R, D y la interacción R x D. Mientras que el porcentaje de Ovulación y el tamaño del cuerpo lúteo favorecieron (P<0.05) al HSR (96%; 1.04 ± 0.07 cm), se produjo también un aumento (P<0.05) en el tamaño de camada en D350 vs. D100 (2.06 ± 0.2 vs. 1.36 ± 0.2). Las variables EI, LAT, DUR, OR y PREG se vieron afectados por la interacción R x D. En el segundo experimento, evaluamos la posibilidad de reducir la dosis de eCG para inducir la actividad reproductiva en cabras anéstricas. Se utilizaron 39 cabras criollas de un sistema extensivo, ubicadas en cuatro grupos (eCG-200 n=10, eCG-100 n=10, eCG-50+50 y eCG-50 n=9). Se aplicó 25 mg de progesterona y 24 h después se administró la eCG: 200 UI al eCG-200, 100 UI al eCG-100, 50 UI al eCG-50+50 y 12 h posteriores otra dosis de 50 UI y 50 UI al eCG-50. Los porcentajes de hembras en estro y de ovulación, fueron mayores al 75% en todos los grupos. Los porcentajes de preñez fueron iguales (P>0.05) en los grupos eCG-200, eCG-100 y eCG-50+50, pero diferentes (P<0.05) a eCG-50. Por lo tanto, los resultados de esta investigación muestran fundamentos para definir estrategias en la inducción de la actividad reproductiva en cabras anéstricas.

Palabras claves: cabra, rango social, dosis de eCG, anestro estacional, protocolo hormonal, inducción del estro y ovulación.

ABSTRAC

We evaluated the effect of the social rank [R] (low-LSR, medium-MSR or high-HSR) in anestrous goats, which were subjected to a estrus induction protocol (EIP) based on progesterone + eCG [D] (100 or 350 IU). Seventy (70) goats managed under an intensive system were used. The effect of R, D and the R x D interaction were evaluated. While the ovulation percentage and the corpus luteum size favored the HSR (96% 1.04 ± 0.07 cm), there was also an increase for litter size for D350 vs D100 (2.06 ± 0.2 vs. 1.36 ± 0.2). The EI, LAT, DUR, OR and PREG variables were affected by the R x D interaction. For the second experiment, we evaluated the possibility of reducing the eCG dose in order to induce reproductive activity in anestrous goats. We used 39 creole goats managed under an extensive system, an allocated in 4 groups (eCG-200 n=10, eCG-100 n=10, eCG-50+50 and eCG-50 n=9). Progesterone (25 mg) was administered 24 h after the eCG administration: 200 IU to eCG-200, 100 IU to eCG-100, 50 IU to eCG-50+50 and 12 h afterwards, a second dose of 50 IU and 50 IU to eCG-50. The percentage of females in estrus and ovulation were higher than 75% in all groups. The percentage of pregnancy were equal (P>0.05) for groups eCG-200, eCG-100 and eCG-50+50, but different (P<0.05) to eCG-50. Therefore, the results of this research show foundations to define strategies in the induction of reproductive activity in anestrus goats.

Keywords: goats, social ranks, eCG doses, anestrus season, hormonal protocol, estrus and ovulation induction.

INTRODUCCIÓN

Algunos mamíferos presentan un periodo de estacionalidad en su actividad reproductiva, con el propósito de seleccionar la época del año más favorable para sus partos (Bronson, 1985; Bronson y Heideman, 1994). En este sentido, la producción caprina se enfrenta al periodo de anestro estacional que presentan las diferentes razas. Por ejemplo, las cabras de la Comarca Lagunera (26°N), manifiestan su periodo de inactividad sexual entre el mes de marzo al mes de agosto (Duarte *et al*., 2010). Lo anterior, ha ocasionado que los productos obtenidos de las cabras, la leche y el cabrito, se comporten también de manera estacional; minimizando así los recursos económicos obtenidos en este sector productivo (Delgadillo, 2011). Diversos trabajos científicos, se han centrado para contrarrestar el efecto negativo de la estacionalidad reproductiva en los caprinos. Tal es el caso de los protocolos a base de progestágenos, los cuales son administrados en esponjas intravaginales y dispositivos internos de liberación controlada (CIDR). Aunado a estos tratamientos, se utiliza la gonadotropina coriónica equina (eCG) que asegura una óptima respuesta estral y una tasa ovulatoria aceptable (Vilariño *et al.,* 2011; Abecia *et al*., 2012). Sin embargo, estos protocolos presentan grandes inconvenientes, como la presencia de infecciones en el tracto genital de la hembra, tratamientos de larga duración (altas cantidades de hormonas), asimismo, necesidad de mano de obra calificada, instalaciones y equipos adecuados, que vienen a elevar los costos de producción (Viñoles *et al*., 2001; Rubianes y Menchaca 2003). Debido al bienestar animal y en la salud pública, se está cuestionando el uso de dosis elevadas de hormonas exógenas, por lo que se ha planteado el uso razonado de las mismas. Un ejemplo de ello, es la investigación de Contreras-Villarreal *et al*. (2015), quienes aplicaron 25 mg de progesterona intramuscular y 24 horas después una dosis de 250 UI de eCG, induciendo el estro y la ovulación del 100% de las hembras tratadas.

Sin embargo, algunos factores como las relaciones sociales, podrían modificar la respuesta sexual de las hembras caprinas a estos tratamientos. Por ejemplo, el rango social puede afectar la actividad reproductiva en los pequeños rumiantes, tanto en machos como en hembras (Côté, 2000; Pelletier y Festa-Bianchet, 2006;

Aguirre *et al*., 2007; Álvarez *et al*., 2007). Tal es el caso, en las cabras anovulatorias expuestas al efecto macho, se encontró que las hembras dominantes ovularon significativamente antes que las hembras subordinadas. Lo anterior puede ser debido, a un mayor contacto entre las cabras dominantes y el macho (Álvarez *et al*., 2003). Por otra parte, en cabras de Cachemira, al ser estimuladas sexualmente por los machos, se observó que las hembras dominantes respondieron en las primeras 4 horas con una mayor secreción de LH en comparación con las cabras de rango social bajo (Álvarez *et al*., 2007).

En el caso de las cabras domésticas, existen pocas evidencias que describan la relación del rango social sobre la actividad reproductiva de estos animales. En efecto, las investigaciones encontradas van dirigidas a explicar cómo influye el peso corporal, la presencia de cuernos y la edad, sobre el rango social. Con tal evidencia, esta investigación pretende determigar la influencia del rango social sobre la inducción a la actividad reproductiva en cabras anéstricas, tratadas con un protocolo hormonal a base progesterona intramuscular más eCG. Además, comprobar si una dosis de 100 UI de eCG es suficiente para estimular la actividad reproductiva en términos de conducta estral, respuesta ovulatoria y gestación.

Hipótesis generales

1. La actividad reproductiva de las cabras inducida por la aplicación de eCG, podrá ser mayor en las hembras de alto rango social que en los individuos de rangos sociales inferiores.
2. La actividad reproductiva de las cabras en anestro estacional, podrá ser inducida con una dosis de 100 UI de eCG.

Objetivos generales

1. Determinar si el rango social afecta la respuesta reproductiva de las cabras anéstricas al ser sometidas a un tratamiento hormonal de eCG.
2. Evaluar las dosis de eCG en su eficacia para inducir la actividad reproductiva en cabras anéstricas

REVISIÓN DE LITERATURA

Relevancia del ganado caprino en México

Desde el punto de vista productivo, la cabra es una de las especies más importantes para el ser humano, ya que son capaces de producir leche y carne en los diferentes ecosistemas del mundo (Escareño *et al*., 2012).

México, es el segundo país del continente americano como productor de leche de cabra (FAOSTAT, 2013), con una producción de 156 mil toneladas de leche anual y un censo de 8.6 millones de cabezas (SIAP, 2015).

La caprinocultura en México, es practicada básicamente por familias rurales de escasos recursos, concentrándose en las regiones áridas y semiáridas del país. Este sistema de producción depende del pastoreo en tierras comunales, en donde las cabras basan su alimentación todo el año (Mellado *et al*., 2003; Echavarría *et al*., 2006). Además de lo anterior, presenta poco nivel de tecnificación y asesoría especializada, por lo que la productividad es baja, sin embargo, sigue siendo una contribución importante para el sustento de los agricultores (Echavarría *et al*., 2006; Escareño Sánchez *et al*., 2011).

Estacionalidad reproductiva de la cabra

La mayoría de los ecosistemas del mundo presentan variaciones estacionales en el clima y subsecuentemente en la disposición del alimento. A medida que estos hábitats se alejen del Ecuador las variaciones estacionales son más marcadas. Con tal evidencia, podemos conceptualizar la estacionalidad reproductiva como un mecanismo de algunos mamíferos de adaptación a su medio ambiente, con el fin de que los partos se presenten en el mejor momento del año y las crías puedan sobrevivir (Bronson, 1985; Bronson y Heideman, 1994).

Esta estacionalidad reproductiva está regulada por la glándula pineal, quien se encarga de traducir la información fotoperiódica a través de su secreción de la hormona melatonina (Arendt, 1998). En los días cortos del otoño e invierno, aumentan el tiempo de liberación de melatonina, que en la cabra estimula la secreción de la hormona liberadora de gonadotropinas (GnRH) del hipotálamo.

Esta retroalimentación positiva en la liberación de GnRH, provoca cambios correspondientes en la secreción de la hormona folículo estimulante (FSH) y la hormona luteinizante (LH), que son responsables del desarrollo folicular, la ovulación y el crecimiento del cuerpo lúteo (Blaszczyk *et al*., 2004). En este sentido, podemos observar en la cabra Malagueña y la Alpina de latitudes altas (latitudes >40°), que durante el otoño y el invierno despliegan su actividad estral y ovulatoria (Chemineau *et al*., 1992; Gómez-Brunet *et al*., 2010). Mientras que en las cabras nativas de Argentina (latitud 30° Sur), el periodo de anestro estacional ocurre de octubre a enero (primavera-verano) y la actividad sexual de estas cabras se presenta de febrero a septiembre [otoño-invierno] (Rivera *et al*., 2003). Por otra parte, en las cabras de la Comarca Lagunera del subtrópico mexicano (latitud de 26° Norte), presentan su periodo reproductivo de septiembre a febrero, así mismo, el periodo de inactividad sexual está definido del mes marzo al mes de agosto (Duarte *et al*., 2010).

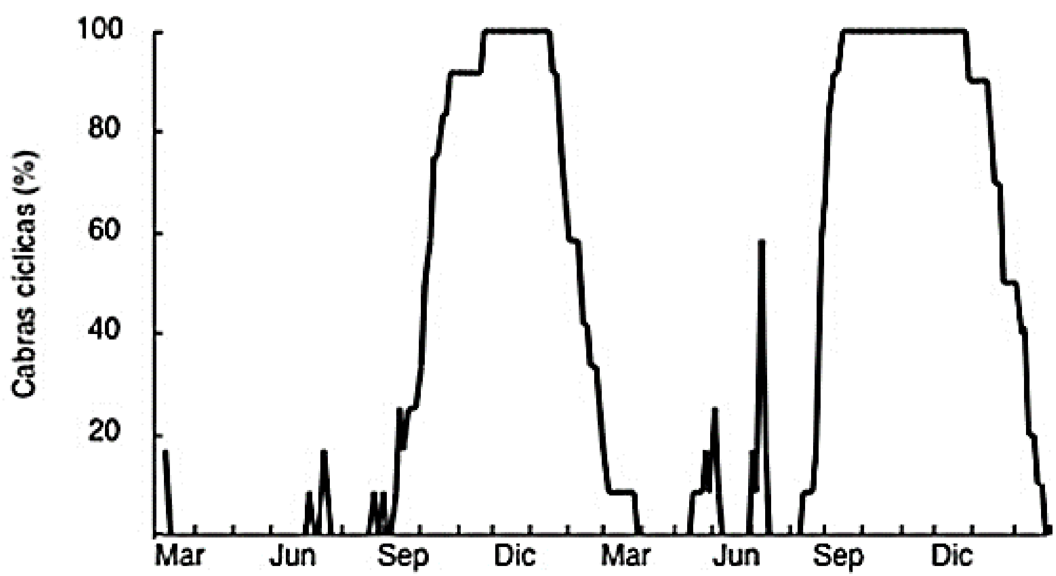

Figura 1. Variaciones estacionales de la actividad ovulatoria en hembras caprinas del subtrópico mexicano (latitud 26°N; adaptado de Duarte *et al*., 2010).

Ciclo estral de la cabra

Las cabras son consideradas como poliéstricas estacionales, ya que presentan varios ciclos estrales durante el periodo de actividad reproductiva (Rivera-Lozano *et al*., 2011), con excepción de las cabras del trópico que presentan ciclos estrales continuos durante todo el año (Chemineau, 1993). El ciclo estral lo podemos definir como el lapso de tiempo entre dos estros, en donde la hembra sufre modificaciones hormonales, anatómicas y de comportamiento. En la cabra, el ciclo estral tiene un promedio de 21 días y clásicamente se ha divido en dos fases: la fase folicular y la fase lútea, cada fase está caracterizada por una hormona dominante y una respectiva estructura ovárica (Fatet *et al*., 2011). Así mismo, la fase Folicular está subdividida en proestro y estro. El proestro empieza con la regresión del cuerpo lúteo y una disminución drástica de la Progesterona (Bono *et al*., 1983; de Castro *et al*., 1999). Subsecuentemente, por la liberación de la FSH en la adenohipófisis se inicia un rápido crecimiento folicular, teniendo como resultado un aumento en la síntesis y secreción del estradiol (Medal *et al*., 2003). Cabe mencionar, que la duración del proestro tiene un promedio de 3 días (Fatet *et al*., 2011). Se define al estro como el periodo de receptibilidad sexual de la hembra hacia el macho. En estos momentos, la hembra presenta el pico más elevado de estradiol, desencadenando los comportamientos típicos del estro en la hembra (Fabre-Nys y Gelez, 2007; Saïd *et al*., 2007). Asimismo, en este periodo se presentan los folículos dominantes listos para la ovulación. Es importante mencionar, que por efecto de la retroalimentación positiva del estradiol sobre la producción de GnRH, provoca el pico preovulatorio de LH y desencadenar así, la ovulación. La expulsión del ovocito se presenta de 9 a 37 horas después de haber iniciado el estro (Saïd *et al*., 2007; Rahman *et al*., 2008; Fatet *et al*., 2011). El estro tiene una duración promedio de 3 días (Fatet *et al*., 2011). De la misma manera, la fase lútea se subdivide en metaestro y diestro (Fatet *et al*., 2011). El metaestro inicia con la ovulación y tiene una duración de 2 a 5 días. Posteriormente a la ovulación la secreción de estradiol empieza a disminuir y la estructura ovárica resultante es el cuerpo hemorrágico, en donde las células de la granulosa y de la teca interna empiezan a unirse para dar lugar al tejido del cuerpo lúteo. En este momento del ciclo estral, la producción de progesterona se realiza en bajas cantidades (Fatet *et al*., 2011). El diestro se caracteriza por ser la etapa más larga del ciclo estral y en donde el cuerpo lúteo es totalmente funcional; por lo que la secreción de progesterona alcanza su pico más elevado. Asimismo, la progesterona tiene una retroalimentación negativa sobre la liberación de gonadotropinas (FSH y LH) con el fin de inhibir la ovulación. Posteriormente, si la implantación del embrión no se realizó exitosamente, el endometrio empezará a secretar prostaglandinas f2α para la lisis del cuerpo lúteo y empezar un nuevo ciclo estral. Es importante mencionar, que la duración del diestro es de 12 días en promedio (Fatet *et al*., 2011; Figura 2).

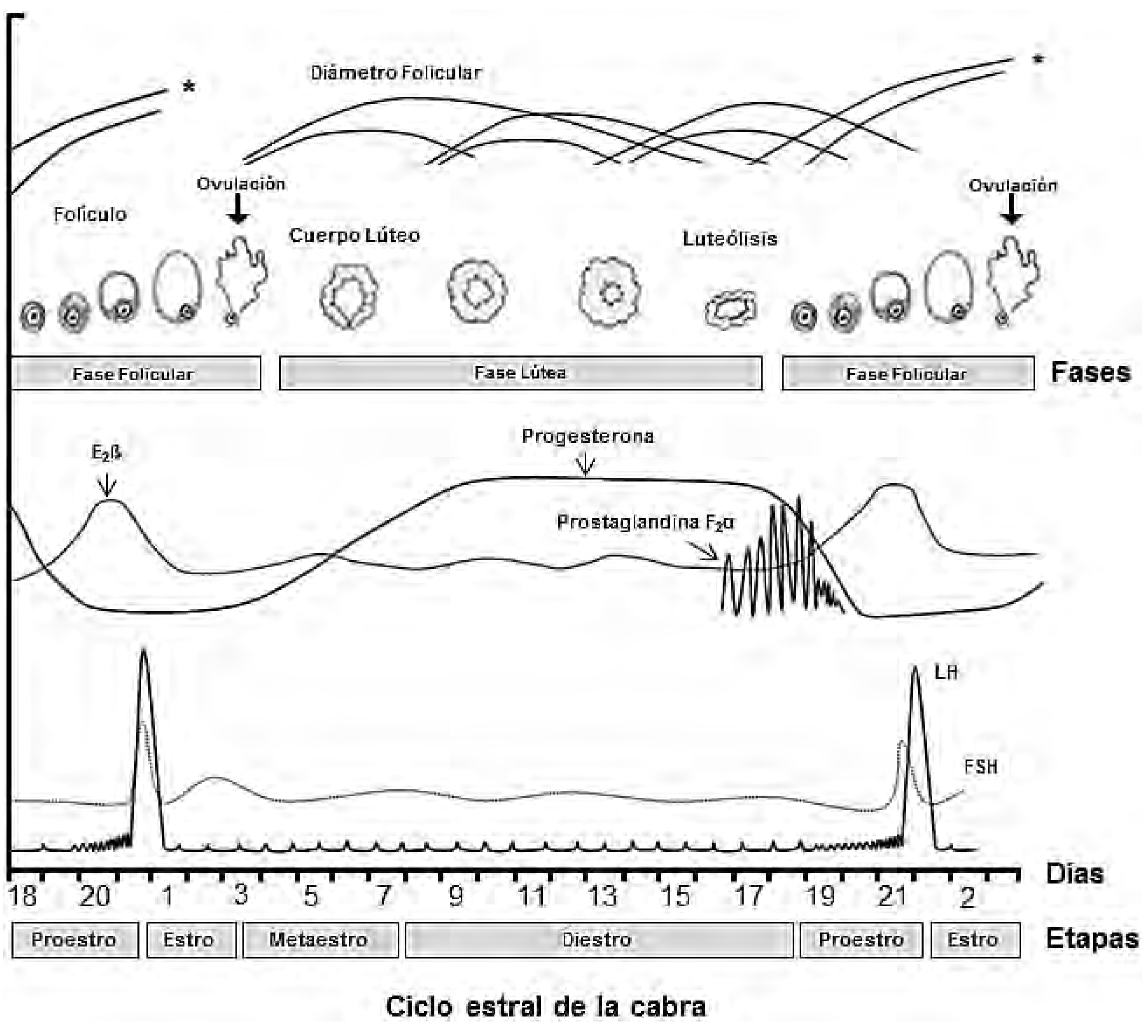

Figura 2. Representación esquemática de los eventos fisiológicos que ocurren durante el ciclo estral de la cabra (Adaptado de Fatet *et al*., 2011).

Tratamientos hormonales para inducir la actividad reproductiva en la cabra

Progestágenos

Los análogos de progesterona con más utilidad para sincronizar e inducir el estro, es el acetato de fluorogestona (FGA) y la medroxiprogesterona (MAP) aplicados por medio de esponjas intravaginales (Romano, 2004; Holtz, 2005). Otras formas de administrar la progesterona es la utilización de presarios vaginales en forma de "Y" (CIDR; Vilariño *et al*., 2011), implantes subcutáneos de liberación lenta (Holtz, 2005) y la más actual, de forma intramuscular (Contreras-Villarreal *et al*., 2015). Cuando se empezaron a utilizar los tratamientos a base de progesterona, tenían una duración de 21 días, simulando la duración promedio de un ciclo estral (Corteel, 1975). En consecuencia, estos tratamientos de larga duración tienden a repercutir en la fertilidad de las hembras (Viñoles *et al*., 2001; Menchaca y Rubianes, 2004). Por lo tanto, en la actualidad se han elaborado protocolos hormonales que han reducido la cantidad de progesterona empleada, minimizando así, su efecto negativo sobre la fertilidad (Vilariño *et al*., 2011; Rodríguez-Martínez *et al*., 2013; Contreras-Villarreal *et al*., 2015).

Gonadotropina coriónica equina (eCG)

En conjunto con la aplicación de cualquier progestágeno, se han utilizado aplicaciones de eCG con el fin de asegurar el crecimiento folicular y posteriormente la ovulación (Fonseca *et al*., 2005; Vilariño *et al*., 2011; Contreras-Villarreal *et al*., 2015). En este sentido, la eCG presenta una dualidad en su actividad biológica, funciona primordialmente como hormona folículo estimulante (FSH) y de forma secundaria como hormona luteinizante (LH; Murphy, 2012; Simões, 2015). En efecto, esta gonadotropina tiene la capacidad de unirse a los receptores de LH y de FSH localizados en las células tecales y de la granulosa, respectivamente (Murphy, 2012). Cabe mencionar que la vida media de la eCG es relativamente larga, lo que potencializa sus efectos a nivel ovárico (McIntosh *et al*., 1975). Con las evidencias, la eCG puede estimular el crecimiento folicular, la secreción de estradiol y subsecuente la manifestación de la actividad estral (De Rensis y López-Gatius, 2014). Es en este momento, que la retroalimentación positiva del estradiol desencadena el pico preovulatorio de LH y posteriormente inducir la ovulación (McNatty *et al*., 1984; Sheldon y Dobson 2000; Murphy, 2012). La eCG por su efecto luteotrófico, promueve el crecimiento del cuerpo lúteo y su funcionalidad (Thatcher *et al*., 2001). En el trabajo de García-Pintos y Menchaca (2016), observaron como la eCG favoreció el crecimiento del área luteal y subsecuente la secreción de progesterona.

La aplicación de la eCG, se puede hacer inmediatamente al retirar los dispositivos intravaginales o 24 horas después de haber suministrado la progesterona intramuscular. Las dosis utilizadas van de 200 hasta 1000 UI, dependiendo de la raza, la edad y la temporada de aplicación (Fonseca *et al*., 2005; Vilariño *et al*., 2011; Abecia *et al*., 2012). Cabe mencionar, que estos protocolos hormonales tienen grandes inconvenientes como afectaciones a la pared vaginal, necesidad de mano de obra calificada, instalaciones y equipos adecuados (Viñoles *et al*., 2001; Rubianes y Menchaca 2003; Manes *et al*., 2015).

Es importante para la producción y para la salud pública, la disminución de las hormonas exógenas (Simões, 2015), un ejemplo de ello es la investigación de Contreras-Villarreal *et al*. (2015), quienes aplicaron una dosis única de 25 mg de progesterona por vía intramuscular y 24 h después administraron 250 UI de eCG, induciendo el 100% del estro y la ovulación de cabras en anestro estacional.

La jerarquía social

A los caprinos se les considera como una especie altamente sociable y gregaria, tanto en vida silvestre como en estabulación (Founier y Festa-Bianchet, 1995; Côté, 2000; Álvarez *et al*., 2003). Considerando lo anterior, existen ventajas de vivir en sociedad, como son: la protección contra los depredadores, la defensa del territorio ocupado y una mayor probabilidad de encontrar alimento y compañeros sexuales (Craight, 1981; Broom y Fraser, 2007). En contraparte, también existen desventajas de vivir en sociedad, por ejemplo: contraer enfermedades infecciosas, distribución imparcial de los recursos como son agua, alimento, y lugares de descanso (Founier y Festa-Bianchet, 1995; Côté, 2000; Ungerfeld y Correa, 2007).

Podemos describir a la jerarquía como la estructura social del grupo, donde encontramos animales dominantes y animales subordinados (Craig, 1981; Barroso *et al*., 2000; Álvarez *et al.,* 2003). En este sentido, el rango social es la posición que ocupa un individuo dentro de la estructura social (Côté, 2000; Pelletier y Festa-Bianchet, 2006), donde el animal dominante corresponde a un rango alto y el subordinado a un rango bajo (Côté, 2000). Cabe mencionar que el rango social es definido básicamente por el peso corporal, la presencia de cuernos y la edad (Barroso *et al*., 2000; Côté, 2000; Pelletier y Festa-Bianchet, 2006).

Efecto del rango social sobre la reproducción

Existen trabajos de investigación que describen el efecto que tiene el rango social sobre la actividad reproductiva en pequeños rumiantes. En efecto, los individuos de alto rango social tienen mayor éxito reproductivo en general, que los animales de rangos sociales inferiores (Côté y Festa-Bianchet, 2001; Pelletier y Festa-Bianchet, 2006; Pluháček *et al*., 2006; Aguirre *et al*., 2007; Álvarez *et al*., 2007; Dušek *et al*., 2007). Tal es el caso del ciervo rojo (*Cervus elaphus*), donde las hembras dominantes se gestantes anticipadamente que las hembras subordinadas (Clutton-Brock *et al*., 1986). Otro ejemplo son las cabras de montaña (*Oreamnos americanus*), donde las madres dominantes tuvieron un mayor éxito reproductivo en la producción de cabritos, en comparación con el número de nacidos de las madres subordinadas (Côté y Festa-Bianchet, 2001). En este sentido, en las hembras del íbice español (Capra pyrenaica) en cautiverio, se encontró que las cabras dominantes tuvieron un mayor número de ciclos estrales durante la época reproductiva, como también un mayor porcentaje de preñez, comparadas con las cabras subordinadas (Santiago-Moreno *et al*., 2006).

En cabras domesticadas sometidas al efecto macho, las hembras dominantes respondieron en las primeras 4 horas con mayores pulsos de LH y manifestaron mayor éxito en la expresión del celo (Álvarez *et al*., 2007). Además, en las cabras expuestas a la bioestimulación sexual por los machos, se ha demostrado que las hembras de alto rango social ovulan y se gestan, significativamente antes que las cabras del rango social bajo. En efecto, se ha descubierto que las cabras dominantes tienen un mayor contacto con los machos, pudiendo de esta manera favorecer la bioestimulación sexual y por lo tanto inducir la actividad reproductiva anticipadamente (Álvarez *et al*., 2003). Un mayor contacto durante el efecto macho, tendrá como resultado una mayor secreción de la hormona luteinizante (LH), hecho sucedido en las cabras dominantes (Álvarez *et al*., 2007; 2009).

Álvarez *et al*. (2010) después de sincronizar la ovulación, encontraron que las cabras del rango social alto tuvieron una mayor secreción de progesterona durante todo el estudio, con respecto a los otros rangos sociales. Además, esta investigación encontró que, durante el reconocimiento temprano de la gestación, las cabras del rango social alto presentaron mayores niveles plasmáticos de progesterona. Asimismo, en un programa de superovulación, se encontró que las cabras dominantes presentaron un mayor número de cuerpos lúteos (Ungerfeld *et al*., 2007).

Por otra parte, en estudios con machos cabríos durante la época reproductiva, los animales de alto rango social presentan mayores concentraciones de testosterona, un mayor volumen de semen eyaculado y mejores concentraciones espermáticas que los animales subordinados (Aguirre *et al*., 2007). En el caso de los carneros, los machos dominantes presentan más montas exitosas que los machos de rango social bajo. Además, los carneros de rango social alto restringen en su actividad reproductiva a los carneros de rangos sociales inferiores (Hulet *et al*., 1962). Se ha comprobado en corderos, que los animales dominantes llegan anticipadamente a la pubertad en relación a los corderos subordinados. Además, los corderos dominantes despliegan un mejor comportamiento sexual cuando son expuestos a las hembras (Ungerfeld y González-Pensado, 2008).

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ESTUDIO 1

ARTÍCULO 1: Effect of social rank upon estrus induction and some reproductiveoutcomes in anestrus goats treated with progesterone + eCG.

animals

MDPI

Article

Effect of Social Rank upon Estrus Induction and Some Reproductive Outcomes in Anestrus Goats Treated With Progesterone + eCG

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Simple Summary: The potential effect of social rank [R] (high—HSR; medium—MSR; low—LSR) in anestrus goats subjected to an estrus induction protocol (EIP) primed with progesterone (P4) and receiving a differential equine chorionic gonadotropin (eCG) dose [D] (D100 vs D350) upon some reproductive outcomes in crossbred dairy goats under intensive stall-fed conditions was evaluated. Response variables included estrus induction (EI, %), latency to estrus (LAT, h), duration of estrus (DUR, h), ovulation (OVU, %), ovulation rate (OR, n), corpus luteum size (CLS, cm), pregnancy (PREG, %), kidding (KIDD, %), and litter size (LS, n). Most of the response variables were positively affected by social rank, favoring to the HSR goats (i.e., EI %, DUR h, OVU %, OR n, and CLS cm). In addition, increased OR and PREG occurred in the HSR + D350 group, while D350 increased LS, irrespective of R. Interestingly, since no differences regarding LAT, DUR, OVU, CLS, PREG, and KIDD occurred between D350 and D100, the obtained values support the use of a reduced level of exogenous hormones to induce and generate out-of-season reproductive efficiency.

Abstract: We evaluated the possible role of the social rank [R] (i.e., low—LSR, middle—MSR, or high—HSR) in anestrus goats exposed to a P4 + eCG [D] (i.e., 100 or 350 IU) estrus induction protocol (EIP). Adult, multiparous (two to three lactations), multiracial, dairy-type goats (Alpine–Saanen–Nubian x Criollo goats ($n = 70$; 25°51′ North) managed under stall-fed conditions were all ultrasound evaluated to confirm anestrus status while the R was determined 30 d prior to the EIP. The variables of estrus induction (EI, %), estrus latency (LAT, h), estrus duration (DUR, h), ovulation (OVU, %), ovulation rate (OR, n), corpus luteum size (CLS, cm), pregnancy (PREG, %), kidding (KIDD, %), and litter size (LS, n) as affected by R, D, and the R × D interaction, were evaluated. While OVU and CLS favored ($p < 0.05$) HSR (96% and + 1.04 ± 0.07 cm), an increased ($p < 0.05$) LS occurred in D350 vs. D100 (2.06 ± 0.2 vs. 1.36 ± 0.2); neither R nor D affected ($p > 0.05$; 38.5%) KIDD. However, EI, LAT, DUR, OR, and PREG were affected by the R × D interaction. The HSR group had the largest ($p < 0.05$) EI % and DUR h, irrespective of D. The shortest ($p < 0.05$) LAT occurred in

D350, irrespective of R. While the largest ($p < 0.05$) OR occurred in HSR and MSR within D350, the HSR + D350 group had the largest PREG ($p < 0.05$). These research outcomes are central to defining out-of-season reproductive strategies designed to attenuate seasonal reproduction in goats.

Keywords: goats; social ranks; anestrus season; estrus induction protocol; reproductive efficiency

1. Introduction

The goat is a gregarious species [1–3] with a well-defined hierarchical structure and different social ranks coexist [2,4]. These social behavioral differences give individuals different opportunities not only to survive [2,3,5], but to express reproductive fitness and success in small ruminants, in either males or females [2,4,6,7]. Indeed, in mountain goats (*Oreamnos americanus*), the dominant females displayed a greater reproductive success regarding the number of kids born as compared to the subordinate goats [8]. Furthermore, in females of the Spanish ibex (*Capra pyrenaica*) in captivity, the dominant goats exhibited a greater number of estrus cycles and higher pregnancy rates, as compared to the subordinate goats [9]. Certainly, when exposed to the male effect, the hierarchically dominant Kashmir female goats responded in the first 4 h with not only higher luteinizing hormone (LH) pulses but also greater success when expressing estrus [7]. Furthermore, high socially ranked female goats exposed to the male effect had a significantly earlier ovulation and greater pregnancy rates compared to low socially ranked goats. Indeed, hierarchically dominant goats had longer contact periods with males, which were thus able to promote an enhanced sexual bio-stimulation and, therefore, induced increased reproductive outcomes [1]. Certainly, a longer male-to-female interaction during the male effect process promotes an increased secretion of luteinizing hormone (LH), mainly in dominant goats [7,10]. Moreover, when exposed to an estrus synchronization protocol, high-ranking goats had higher progesterone secretion during the maternal recognition stage of the pregnancy process relative to low or medium social ranks [11]. Similarly, in a superovulation protocol, the dominant goats presented a greater number of corpus lutea [12].

On the other hand, the goat is considered a seasonal short-day polyestrus breeder, which allows it to mate and give birth in a defined season of the year [13,14]. This reproductive seasonality causes milk, cheese, and meat to concentrate in some specific months of the year, causing economic losses to both producers [15] and industrializers [16,17]. To resolve this problem and to induce out-of-season sexual activity, diverse estrus-inducing hormone protocols have been used, obtaining interesting results regarding both estrus induction and ovulation [18]. Among the hormonal protocols to control reproductive activity in goats, are those progestogen-based protocols involving natural progesterone [19], fluorogestone acetate [20], or medroxyprogesterone acetate [21]. Those estrus induction protocols using progestogens are normally accompanied by the use of gonadotropins, such as human chorionic gonadotropin (hCG) [22] or equine chorionic gonadotropin (eCG) [18].

Currently, animal production is aimed at sustainability, with a strict regulation regarding the use of exogenous hormones [18]. Therefore, any attempt to decrease the use of exogenous hormones for reproductive control is not only an interesting option but also a claimed area of research. The use of 100 IU of hCG has been shown to effectively induce reproductive activity in anestrus goats, obtaining similar results with conventional higher doses [12,23]. Nonetheless, in goats, there is little evidence regarding the relationship between social rank and the induction of sexual activity in goats subjected to a hormonal estrus-inducing protocol. Building on such findings, we hypothesized a differential response according to social rank in anestrus goats subjected to an intramuscular progesterone + eCG estrus induction protocol; while a better reproductive response is expected to occur in the HSR goats, we also propose that a similar reproductive performance will be observed in goats receiving either a high or a low eCG dose (i.e., 100 or 350 IU); this study aimed to answer such an inquiry.

2. Material and Methods

2.1. General

All the experimental procedures, methods, and the management of the trial experimental units used in this study were compliant with the guidelines for the ethical use, care, and welfare of animals in research at international [24] and national [25] levels, with institutional approval reference number UAAAN-UL-18-3059.

2.2. Location, Environmental Conditions, Animals, and their Management

The study was carried out in the Comarca Lagunera, Coahuila, northern Mexico (25°51′ North, 103°16′ West, 1190 m), during February and March of the natural anestrus season at this latitude [19,22]. Adult, multiparous (two to three lactations), multiracial, dairy-type goats (Alpine–Saanen–Nubian × Criollo; n = 70) managed under intensive, stall-fed conditions were distributed into two homogeneous groups regarding live weight (LW, 41.9 ± 9.08 kg) and body condition (BC, 1.87 ± 0.04; scale from 1 to 4 [26]). The groups were housed in two pens with an area of 80 m^2 each; fodder was provided three times a day (08:00, 13:00, and 17:00), including alfalfa hay and 200 g per goat/day of commercial concentrate (14% CP). Water and mineral salts were provided ad libitum. During the pre-trial stage, the anestrus status of goats was confirmed through two trans-rectal ultrasound scans, using a 7.5 MHz human prostate transducer (Aloka 500, MHz linear array; Corometrics Medical Systems, Inc., Wallingford, CT, USA). Prior to the ultrasound scan, the transducer was lubricated and then inserted into the goat's rectum to determine the type of ovarian structures present in both ovaries. Goats with the presence of corpus lutea were discarded from the study. The central activities performed during the experimental period are depicted in Figure 1.

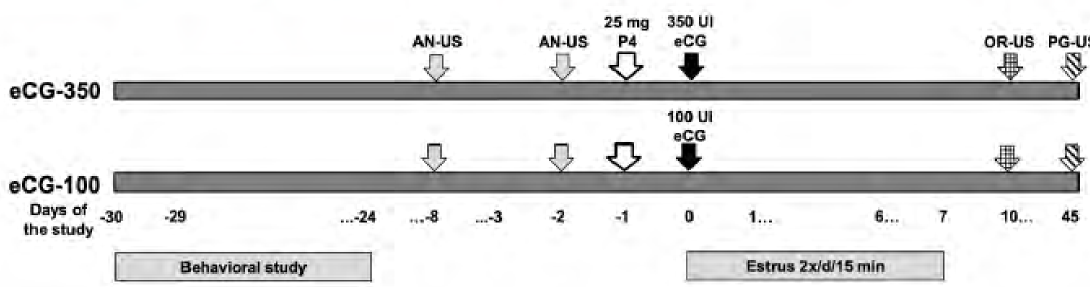

Figure 1. Schematic representation of the experimental protocol. In February, a behavioral study was carried out to define the social ranks: high (HSR), middle (MSR), or low (LSR) social rank. Then, all goats were exposed to an estrus induction protocol, in order to induce reproductive activity during the natural anestrus season in Northern Mexico (March; 25°51′ North). All goats were primed with progesterone (P4) and received different doses of equine chorionic gonadotropin (eCG) (100 or 350 IU). Estrus activity was evaluated daily after the application of eCG doses up to day 7. Transrectal ultrasound (US) scanning was performed on days −8 and −2 to confirm anovulation (AN) as well as on days 10 and 45 post eCG treatment, to assess both ovulatory rate (OR) and pregnancy rate (PG), respectively.

2.3. Behavioral Study, Social Rank, Treatment Groups, Measurements, and Response Variables

To determine the goat's social rank, one month prior to the treatment group formation (i.e., the application of the eCG), a behavioral study was carried out in February as previously outlined [1]. The behavioral test was performed at feeding time (08:00, 13:00, and 17:00) during a 60 min period during the 7 days pre-trial period. Therefore, the main interactions exerted among breeding female goats were monitored for 180 min d^{-1}, for a total of 1260 min (i.e., 21 h) during the whole pre-trial behavioral study. The following behavioral goat-to-goat interactions were documented:

bumps, threats, shoves, chases, escapes, and evasions. All the agonistic interactions during each 60 min observation period (180 min per day) were recorded; the abovementioned agonistic interactions between two individuals that involved an instigator or a victim, whether or not physical contact occurred, and that resulted in the physical displacement of an animal, were therefore considered. With the information obtained from the agonistic interactions, that is, the result of either winning or being defeated, a success rate (IE) was calculated considering the following formula: IE = number of individuals able to displace/(number of individuals able to displace + number of individuals displaced). According to the obtained IE, goats were classified into three social ranks: low (LSR; IE 0 to 0.33), medium (MSR; IE 0.34 to 0.66) and high (HSR; IE 0.67 to 1) [1,10]. The ethogram considering the observed social hierarchy according to the average agonistic interactions of the response variables during the behavioral test within each social rank are presented in Table 1.

Table 1. Least square means ± standard error for winning events [threats, bumps, shoves, and chases] or lost events [evasions and escapes] in the behavioral study carried out to define the social hierarchy based on a success index according to the average agonistic interactions defining the low (LSR), medium (MSR), or high (HSR) social rank in multiracial (Alpine–Saanen–Nubian × Criollo; n = 70) dairy goats managed under intensive stall-fed conditions in Northern Mexico (February, 25°51′ North).

Behaviors	Social Rank		
	LSR	MSR	HSR
Threats	25 ± 5.6	106 ± 8.7	231 ± 19.5
Bumps	6 ± 1.4	27 ± 3.6	46 ± 7.7
Shoves	2 ± 0.5	5 ± 1.0	13 ± 3.2
Chases	0 ± 0	0 ± 0	1 ± 0.5
Evasions	345 ± 48.1	125 ± 11.1	67 ± 8.9
Escapes	1 ± 0.4	0 ± 0	0 ± 0
Success Index [1]	0–0.33	0.34–0.63	0.63–1.0

[1] Number of individuals able to displace/(number of individuals able to displace + number of individuals displaced).

Once the anestrus status confirmation and the social rank was established (i.e., LSR, MSR, HSR), the confirmed social rank groups were returned to the pens; fodder was provided three times a day as described. Then, in mid-March, all goats received one intramuscular dose of 25 mg of progesterone (Progesvit®, Brovel, Mexico). One day later, the D100 group (n = 35) received 100 IU of eCG per female (Folligon®, Intervet, Mexico) while, simultaneously, the D350 group received 350 IU of eCG per female. Both progesterone and eCG doses were applied intramuscularly; the LSR, MSR, and HSR were randomly distributed within each eCG dose group.

Thereafter, the estrus activity was monitored daily from the day of eCG application until day 7 of the experimental period (Figure 1); the evaluation of estrus behavior was carried out twice a day (09:00 and 17:00 h) and lasted 15 min for each evaluation. To identify the estrus activity, a total of seven sexually active males were used; in order to prevent sexual intercourse, each buck was aproned. Previously, bucks were subjected to a hormonal treatment of testosterone to activate their sexual behavior and ensure libido [27]. Once the goat remained immobile and allowed the teaser-buck to mount, the onset of estrus was ruled. Subsequently, the apron was removed from the bucks and the female goats from both experimental groups were exposed to natural mounts for the first 12 h after the onset of the estrus.

The percentage of females in estrus was considered as the number of estrus females/total treated females × 100. Latency to estrus was defined as the time elapsed between the application of eCG and the first mount allowed by the goat. The duration of the estrus was considered as the interval between the first and the last mount allowed by the female. Ovulatory activity was measured 10 d after the application of the eCG, by means of a transrectal ultrasound (Figure 1). The percentage of goats that ovulated was considered as the total of females that ovulated/total treated females × 100. The ovulatory rate was defined as the total number of corpus lutea per group compared to the total number of ovulating goats. The pregnancy rate was evaluated by means of a transrectal ultrasound at 45 d after the application of the eCG. Therefore, the response variables included: estrus induction (EI, %), latency to estrus (LAT, h), duration of estrus (DUR, h), ovulation (OVU, %), ovulation rate (OR, n), corpus luteum size (CLS, cm), pregnancy (PREG, %), kidding (KIDD, %), and litter size (LS, n). Due to the fact that the definition of the social rank status in each goat was individually classified, each goat within the eCG dose treatment group was defined as an experimental unit.

2.4. Statistical Analyses

The statistical design was a completely randomized 3 × 2 factorial arrangement with three social ranks (i.e., LSR, MSR, HSR) and two eCG doses (i.e., 100 or 350 IU). The response variables were ANOVA analyzed; the model included the independent variables dose, social rank, and the interaction, with each animal considered as a single experimental unit [28]. In the event of a significant effect, least square mean separation considered the PDIFF option; the analyses were solved by means of the GLM procedures of SAS. Because of their non-normal distribution, categorical variables were analyzed through the CATMOD procedure of SAS. All the analyses were computed through the procedures of SAS (SAS Inst. Inc. Version 9.4, 2016, Cary, NC, USA); the significance level was set at $p < 0.05$.

3. Results

3.1. Effect of Social Rank and the eCG Dose upon the Response Variables

The dependent variables estrus induction live weight (LW, kg), body condition (BC, units), (EI, %), latency to estrus (LAT, h), duration of estrus (DUR, h), ovulation (OVU, %), ovulation rate (OR, n), corpus luteum size (CLS, cm), pregnancy (PREG, %), kidding (KIDD, %), and litter size (LS, n) as affected by the social rank [R] (i.e., HSR, MSR and LSR) and eCG dose [D] (i.e., 100 or 350 IU), as well as the R × D interaction, are shown in Table 2.

Table 2. Least square means ± standard error for live weight (LW, kg), body condition (BC, units), estrus induction (EI, %), latency to estrus (LAT, h), duration of estrus (DUR, h), ovulation (OVU, %), ovulation rate (OR, n), corpus luteum size (CLS, cm), pregnancy (PREG, %), kidding (KIDD, %), and litter size (LS, n) according to social rank (i.e., LSR, MSR, and HSR) and eCG dose (i.e., 100 or 350 mg) in multiracial (Alpine–Saanen–Nubian × Criollo; $n = 70$) dairy goats managed under intensive stall-fed conditions in Northern Mexico (March, 25°51′ North) [1].

Variables	Social Rank (R)			eCG Dose (D)		p Value		
	LSR	MSR	HSR	100	350	R	D	R × D
LW (kg)	31.6 ± 1.6^{b}	44.0 ± 1.3^{a}	49.0 ± 1.4^{a}	41.9 ± 1.5^{a}	41.8 ± 1.4^{a}	0.001	0.615	0.001
BC (units)	1.8 ± 0.07^{a}	1.9 ± 0.06^{a}	1.9 ± 0.06^{a}	1.9 ± 0.05^{a}	1.9 ± 0.05^{a}	0.768	0.733	0.978
EI (%)	10/18 (56) ^{b}	20/28 (71) ^{b}	23/24 (96) ^{a}	26/35 (74) ^{a}	27/35 (77) ^{a}	0.007	0.599	0.019
LAT (h)	57.6 ± 4.6^{a}	69.6 ± 3.2^{a}	68.3 ± 3.0^{a}	76.1 ± 3.0^{b}	57.7 ± 2.8^{a}	0.373	0.001	0.001
DUR (h)	17.3 ± 4.0^{b}	18.9 ± 3.2^{b}	29.0 ± 3.5^{a}	20.2 ± 2.9^{a}	23.6 ± 2.9^{a}	0.004	0.313	0.051
OVU (%)	14/18 ^{b} (78)	26/28 ^{ab} (93)	23/24 ^{a} (96)	30/35 ^{a} (86)	33/35 ^{a} (94)	0.05	0.177	0.294
OR (n)	1.27 ± 0.17^{a}	1.77 ± 0.13^{a}	1.58 ± 0.14^{a}	1.09 ± 0.10^{b}	2.06 ± 0.10^{a}	0.079	0.001	0.001
CLS (cm)	0.8 ± 0.09^{b}	1.05 ± 0.07^{a}	1.04 ± 0.07^{a}	0.91 ± 0.06^{a}	1.05 ± 0.06^{a}	0.046	0.08	0.13
PREG (%)	9/18 ^{a} (50)	14/28 ^{a} (50)	14/24 ^{a} (58)	16/35 ^{a} (46)	21/35 ^{a} (60)	0.651	0.147	0.037
KIDD (%)	5/18 ^{a} (28)	10/28 ^{a} (36)	12/24 ^{a} (50)	11/35 ^{a} (31)	16/35 ^{a} (46)	0.279	0.184	0.119
LS (n)	1.60 ± 0.4^{a}	1.80 ± 0.3^{a}	1.83 ± 0.2^{a}	1.36 ± 0.2^{b}	2.06 ± 0.2^{a}	0.228	0.023	0.139

[1] In February, a behavioral study was carried out to define the social ranks; low (LSR), middle (MSR), or high (HSR) social rank. Then, all goats were exposed to an estrus induction protocol to induce reproductive activity (March). All goats were primed with progesterone (P4) and received different doses of eCG (100 or 350 IU). ^{a,b} Least square means without a common superscript within a response variable are different ($p < 0.05$).

While LW was affected ($p < 0.05$) by social rank, observing the best LW values in both the HSR and MSR, the lowest LW occurred in the LSR and no LW differences ($p > 0.05$) occurred between eCG doses. Moreover, neither R, nor D or even the R × D interaction affected the phenotypic expression of BC. The response variables OVU and CLS favored ($p < 0.05$) the HSR (96% and 1.04 ± 0.07 cm) and MSR (93% and 1.05 ± 0.07 cm) goats, with the LSR goats showing the lowest values (78% and 0.08 ± 0.09

cm). In turn, an increased ($p < 0.05$) LS occurred in the D350 group (2.06 ± 0.2 vs. 1.36 ± 0.2 cm; no differences ($p > 0.05$; 38.5%) in KIDD occurred among social ranks nor between eCG doses.

3.2. Effect of Social Rank × eCG Dose Interaction upon the Response Variables

A rank x dose interaction ($p < 0.05$) affected the response variables LW, EI %, LAT, DUR, OR, and PREG. Therefore, information on such response variables in the HSR, MSR, and LSR as affected by the eCG doses is shown in Table 3. While LW was affected ($p < 0.05$) by the R × D interaction, the best LW values occurred in HSR and MSR and LSR had the lowest LW, the last irrespective of the eCG dose. In addition, the largest ($p < 0.05$) EI % was observed in the HSR goats, irrespective of eCG (i.e., 100 or 350 IU).

Table 3. Least square means ± s.e. for live weight (LW, kg), estrus induction (EI, %), latency to estrus (LAT, h), duration of estrus (DUR, h), ovulation (OVU, %), ovulation rate (OR, n), corpus luteum size (CLS, cm), pregnancy (PREG, %), kidding (KIDD, %), and litter size (LS, n) as affected by the social rank (i.e., HSR, MSR, and LSR) × eCG dose (i.e., 100 or 350 mg) interaction in multiracial (Alpine–Saanen–Nubian × Criollo; $n = 70$) dairy goats managed under intensive stall-fed conditions in Northern Mexico (March, 25°51′ North) [1].

Variables	eCG-100			eCG-350		
	LSR	MSR	HSR	LSR	MSR	HSR
LW (kg)	30.0 ± 2.4 ^{b}	43.4 ± 1.8 ^{a}	47.6 ± 1.9 ^{a}	33.0 ± 2.1 ^{b}	44.7 ± 1.8 ^{a}	47.4 ± 2.1 ^{a}
EI (%)	3/8 ^{c} (38)	11/14 ^{ab} (79)	12/13 ^{ab} (92)	7/10 ^{ab} (70)	9/14 ^{bc} (64)	11/11 ^{a} (100)
LAT (h)	72.0 ± 8.4 ^{abc}	79.6 ± 4.4 ^{a}	74.0 ± 4.2 ^{ab}	51.4 ± 5.5 ^{d}	57.3 ± 4.8 ^{cd}	62.2 ± 4.4 ^{bcd}
DUR (h)	7.5 ± 5.9 ^{b}	18.9 ± 4.5 ^{ab}	29.5 ± 4.6 ^{a}	25.2 ± 5.3 ^{a}	18.9 ± 4.5 ^{ab}	28.4 ± 5.0 ^{a}
OR (n)	0.86 ± 0.3 ^{d}	1.2 ± 0.2 ^{cd}	1.2 ± 0.2 ^{cd}	1.6 ± 0.2 ^{bc}	2.3 ± 0.2 ^{a}	2.1 ± 0.2 ^{ab}
PREG (%)	3/8 ^{b} (38)	9/14 ^{ab} (64)	4/13 ^{b} (31)	6/10 ^{ab} (60)	5/14 ^{b} (36)	10/11 ^{a} (91)

[1] In February, a behavioral study was carried out to define the social ranks; low (LSR), middle (MSR), or high (HSR) social rank. Then, all goats were exposed to an estrus induction protocol to induce reproductive activity (March). All goats were primed with progesterone (P4) and received different doses of eCG (100 or 350 IU). $^{a–d}$ Least square means without a common superscript within a response variable are different ($p < 0.05$).

In general, the greater the eCG dose, the shorter the time it took for estrus to occur; D350 generated the shortest time ($p < 0.05$) for estrus appearance, irrespective of social rank. Regarding the estrus duration (DUR, h), the shortest estrus was shown by the LSR + D100 combination, with no differences among HSR and MSR either with D100 or D350. With respect to ovulation rate, the largest values ($p < 0.05$) were shown by the HSR and MSR within D350, with intermediate values in the HSR, MSR within D100, and LSR + D350; the lowest ($p < 0.05$) OR was shown by the LSR + D100 goats. Regarding the pregnancy rate, the best values ($p < 0.05$) were observed in the HSR + D350, the MSR + D100, and the LSR + D350. Finally, while in the HSR goats the estrus peak occurred 84 h after eCG administration, in the MSR and LSR groups, it occurred at 60 h. With respect to the eCG dose, while the estrus peak in the D350 group occurred 60 h after eCG administration, in the D100 group, it occurred within an interval of 72 to 96 h (Figure 2A,B).

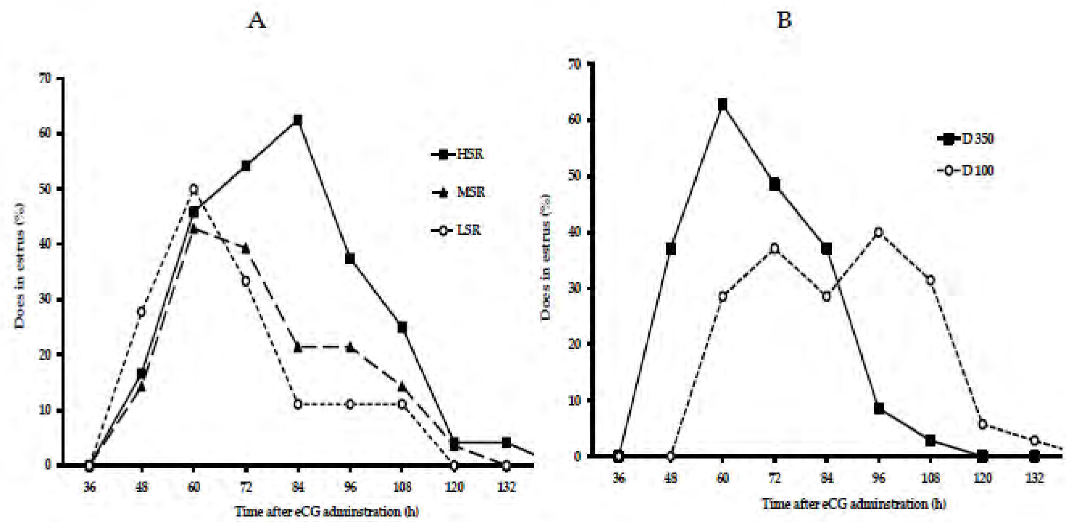

Figure 2. Percentage of estrus induction with respect to the application of equine chorionic gonadotropin (eCG), until the appearance of estrus behavior in goats according to (**A**) social rank (high, medium, and low) and (**B**) eCG dose (100 or 350 IU) in multiracial (Alpine–Saanen–Nubian × Criollo; $n = 70$) dairy goats managed under intensive stall-fed conditions in Northern Mexico (March, 25°51′ North). All goats were primed with progesterone and received different doses of eCG (100 or 350 IU) according to the defined social rank: low, middle, or high.

4. Discussion

The obtained results support our working hypothesis, in that most of the response variables were positively affected by social rank, favoring to the HSR goats (i.e., EI %, DUR h, OVU %, OR n, and CLS cm). In addition, an increased OR and PREG occurred in the HSR + D350 group, and D350 increased LS, irrespective of social rank. Dominance hierarchies encompass animal societies; social rank is affected by either internal (i.e., body size, body weight, body condition) or external (i.e., parental dominance, previous experiences) factors [29]. Certainly, in many ruminant species, reproductive success and access to food are not shared equally among the members of a herd. While social dominance ensures access to the best available food, it also exerts a positive and significant effect upon live weight. In turn, such an increased live weight enhances the metabolic status while boosting reproductive fitness [30]. Moreover, even when free access to food is available, dominant cues would promote the selection of the most nutritive ration because of a preferential access to food, favoring live weight [31]. Energy balance is a key internal cue for an animal to use in order to decide whether or not to trigger the onset or resumption of reproductive function [32]. In turn, the energy balance will affect both live weight and body condition, as well as influencing reproductive and productive performance either at pre-breeding [33] or pre-partum stages [34], both in males or females [35]. Other studies have found that, besides to live weight, the presence of horns and chronological age also influence social rank [2,4,36].

As commented, increases in both live weight and body condition are known factors inducing reproductive function in anestrus females or enhancing sexual and reproductive outcomes in cycling females [37,38]. So, based in the positive relationship among HSR, increased LW, and augmented BC, these three components will be closely aligned to an increased LH pulse frequency pattern. Such a behavioral neuro-endocrine scenario is prone to encouraging an augmented ovarian function. Our findings agree with previous studies where high hierarchy females had a faster and higher response than low hierarchy females regarding LH pulse frequency and estrus activity [7]. Some biological triggers of such increased ovarian function include reproductive hormones such as follicle stimulating hormone (FSH) or LH [32,39,40], metabolic endocrine cues such as leptin [41], insulin-like growth factor-1 [42], insulin–triiodothyronine [43], the somatotropic GH-IGF-1 axis [44], some defined neuroendocrine hints such as the kiss-1, kisspeptin, gpr-54 complex, kisspeptinergic neurons, and GnRH release [32,45], some other genomic signals such as the OCT2, TTF1, and EAP1 hypothalamic genes [32,46], and even specific nutritional molecules such as glutamate [47,48] and β-carotene [40,49].

Another interesting physio-neuro-endocrine scenario is that the HSR goats, who showed the heaviest LW, may have exerted an increased ovarian function using a non-GnRH-dependent pathway, while involving the potential action not only of diverse intra-ovarian but intra-follicular systems (i.e., the insulin–glucose, IGF-1, and leptin metabolic systems) [50]. Besides, both depressed metabolic status and reduced LW, as that observed in the LSR goats, have been shown to decrease the number of primordial, primary, and Graffian follicle populations, leading to a reduced aromatase mRNA expression and diminished FSH and LH receptors, as well as reductions in leptin levels and Ob-R transcripts [51]. Based on such findings, is tempting to suggest that the HSR and high LW goats may have exerted an enhanced steroidogenesis in those follicles with the lowest threshold to the action of FSH. Such a scenario would increase follicle recruitment, augmenting the aromatization from androgens to estrogens, enlarging the formation and size of the antrum, while increasing oocyte quality, ovulation rate, and fertilization rate. All of them are followed by enhancements in luteogenesis, progesterone synthesis, embryonic implantation, and gestation [32]. Interestingly, an HSR has been related to high androgen levels in males [52], while females treated with estradiol or testosterone displayed less submissive behavior compared to those receiving progesterone or placebo [53].

With respect to the eCG dose (i.e., 100 or 350 IU) the goat response was similar LW (41.1 kg), BC (1.9 units), EI (75.5%), DUR (21.9 h), OVU (90%), CLS (0.98 cm), PREG (53%), and KIDD (38.5%). Our results agree with other studies, where hormonal protocols involving intramuscular progesterone + eCG or hCG were used in anestrus goats [19,22]. Indeed, the eCG + P4 treatment induced reproductive activity in non-cycling goats. The latter was certainly due to the particular function of this gonadotropin, which has a primary activity of FSH and secondarily of LH [18,54]. Therefore, it can be assumed that both doses were sufficient to stimulate the preovulatory LH surge in both eCG groups, promoting not only ovulation but an optimum estradiol level required for estrus induction, ovulation, and pregnancy. However, a reduced LAT with increases in both OR and LS were observed in the D350 group. This was probably due to the fact that a higher number of FSH and LH receptors were expressed in the D359 group, promoting an increased steroidogenesis, an augmented aromatization in theca cells, a larger antrum formation, and a faster peak in estradiol levels, as well as an earlier LH surge, all of which merged to an increased follicular recruitment augmenting both OR and LS in the D350 goats [18].

5. Conclusions

This study reveals the key effect of social rank—clearly linked to live weight—upon reproductive outcomes in anestrus goats subjected to an estrus induction protocol primed with progesterone and receiving a differential eCG dose, either 100 or 350 IU, in crossbred dairy goats. Most of the response variables were affected by social rank, favoring to the high social rank goats (i.e., EI %, DUR h, OVU %, OR n, and CLS cm). In addition, a reduced LAT, with increases in OR and LS, occurred in the HSR + D350 group, while D350 increased LS irrespective of social rank. The lowest ranked goats were lighter compared to the MSR and HSR goats, indicating a strong food competition. Interestingly, since no differences regarding EI, DUR, OVU, CLS, PREG, or KIDD occurred between eCG doses (i.e., 100 or 350 IU), the obtained values support the use of a reduced level of exogenous hormones for the induction of estrus during the natural anestrus season. Any attempt to reduce the use of exogenous hormones for the reproductive control of domestic animals will always be welcomed by a highly informed society committed to animal welfare and food safety. These findings are relevant not only from a behavioral, physiological, and wellbeing standpoint, but also acquire productive significance in order to speed up the out-of-season reproductive efficiency of the dairy goat industry.

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ESTUDIO 2

ARTÍCULO 2: Evaluation of different eCG doses + progesterone to induce reproductive activity during the transitional reproductive season in anestrous Creole goats.

Original Research Article

Evaluation of different eCG doses + progesterone to induce reproductive activity during the transitional reproductive season in anestrous Creole goats

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ABSTRACT

Background: Goats from subtropical and temperate latitudes show reproductive seasonality. For this reason, the products obtained from goats also shows the same seasonality, reducing the finances of goat keepers. Several studies have focused on reducing reproductive seasonality through the use of hormonal protocols based on progestogens, in addition to the use of equine chorionic gonadotropin (eCG) hormone, used for its double biological activity that ensures an optimal response to induce estrus and pregnancy in goats. The objective was to assess whether reduced doses of eCG are effective in inducing reproductive activity in anestrous goats. **Methods:** During the transition reproductive period

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(June), mix-breed Creole goats (n=39), were treated with intramuscular progesterone (P4), later, the experimental treatments, consisting in different doses (50, 100, 50+50 or 200 IU) of equine chorionic gonadotropin (eCG) were applied. After the application of the experimental protocols, the reproductive activity of the goats was evaluated. **Result:** This study shows that one dose of 100 IU of eCG effectively induced reproductive behavior in anestrous Creole goats towards the end of the seasonal anestrus. This protocol makes the use of exogenous hormones more efficient, with reduce doses, decreasing expenses and is practical use by goat producers.

Key-words: Anestrus, eCG doses, Estrus and ovulation induction, Goats, Progesterone priming.

INTRODUCTION

Goats from subtropical and temperate latitudes are characterized for showing a marked reproductive seasonality (Duarte *et al*., 2008). For this reason, the supply of products obtained from goats (milk and kid meat) also show the same seasonality, reducing in parallel a continuous economic support by the goat keepers (Delgadillo, 2011). Several studies have centered their attention to decrease such reproductive seasonality through the use of hormonal protocols based on the use progestogens, mainly fluorogestone (FGA; 20-40 mg/sponge) and medroxyprogesterone acetate (MAP; 60 mg/sponge). Besides, controlled internal drug release (CIDR) devices are also used, normally impregnated with 0.30 g of natural progesterone (P_4), (Abecia *et al.,* 2012). The use of these intravaginal devices has been reduced to 5 d (Martemucci and D’Alessandro, 2011). In addition, protocols based on equine chorionic gonadotropin (eCG) have been used and displayed a dual biological activity; its primary action is similar to that of the follicle stimulating hormone (FSH), but it has a second action as luteinizing hormone (LH), (Simões, 2015). The afore mentioned actions assure an optimum response to induce estrus and pregnancy in goats (Simões, 2015; Rahman *et al.,* 2017). In goats, the interval between doses of eCG ranges from 200 to 1000 IU, depending on breed, age and season of the year (Najmi *et al.,* 2011; Abecia *et al.,* 2012). Nonetheless, these hormonal protocols have a major drawback, such as vaginal wall imparment, need for skilled labor, adequate facilities and equipment (Manes *et al*., 2015). When considering both public health, animal wellbeing, and productive sustainability, the use of high doses of exogenous hormones and prolonged administrations are being questioned (Simões, 2015). On this respect, Contreras-Villarreal *et al*. (2016), evaluated the use of a sole dose of 25 mg i.m. progesterone plus 250 IU of eCG 24 h apart, which induces 100% of estrus and ovulation in goats during the seasonal anestrus. Besides, Alvarado-Espino *et al.* (2016) achieved a reduction of the dose of human chorionic gonadotropin (hCG) down to 50 IU, in the induction of reproductive activity in anovulatory goats. This hormonal protocol also considered the use of 20 mg i.m. of progesterone, administered 24 h prior to hCG. For such evidences, the objective of the present study was to assess whether reduced doses of eCG are effective in inducing reproductive activity in anestrous goats.

MATERIALS AND METHODS

Ethics statement: All experimental procedures were compliant with the guidelines for ethical use, care and welfare of animals in research at international

(FASS, 2010) and national (NAM, 2002) levels with institutional approval reference number UAAAN-UL/02-03-1402-2620.

Experimental animals and their management: This research took place in the in Ejido Eureka, Gomez Palacio, Durango, México (25°49' North, 03°23' West) during June 2017, month that corresponds to the transition period from seasonal anestrus to reproductive activity, in goats under an extensive system. Mix-breed Creole goats (n=39) homogeneous by body weight 36.94 ± 7.53 kg and body condition score 2.18 ± 0.29 (Walkden-Brown *et al*., 1997; 1 to 4 scale) were randomly distributed in 35 m^2 pens. Feeding was based on alfalfa hay, water and salts *ad libitum.* In addition to 200 g of a commercial concentrated per goat/day (14% CP). To define anestrus status of goats, two transrectal ultrasounds were performed (Fig. 1) by an experienced operator, using an echograph (Aloka 500) provided with a human prostate transducer of 7.5 MHz (Mhz linear array; Corometrics Medical Systems, Inc., Wallingford, CT, USA). At the beginning of the scanning, the goats were placed in vertical position and the transducer was covered with carboxymethylcellulose as a lubricant. Once the transducer was inserted in the rectum, both ovaries were scanned to determine the presence and type of ovarian structures. The anovulation in the goat was determined by absence of corpora lutea in the two ovaries observed.

Experimental treatments groups: All goats were received one dose of 25 mg of progesterone (Progesvit®, Brovel, Mexico, i.m.) and then were randomly allocated to four experimental groups. Which received 24 h later contrasting i.m. doses of eCG: 1) eCG-200 (200 IU of eCG (Folligon®, Intervet, Mexico); n=10), 2) eCG-100 (100 IU of eCG; n=10), 3) eCG-50+50, (two 50 IU of eCG, 12 h apart, n=10), and 4) eCG-50 (50 IU of eCG; n=9); groups were balanced according to body weight and body condition score (Fig. 1).

Evaluated response variables: During the 7 d subsequent to the eCG administration, estrus activity was evaluated during 15 min twice a day (0900 and 1700 h), through the use of 4 sexually active males, protected with an apron to prevent mating. The male goats were induced to sexual activity by a hormonal treatment based on testosterone using the method described by Luna-Orozco *et al*. (2012). Females were considered in estrus when they allowed to be mated and stayed motionless. To calculate the percentage of females in estrus, the total number of females in estrus divided into total of females treated x 100, was considered. The estrus latency was defined as the time elapsed between the eCG administration and the first mounting allowed by goats. The estrus duration was defined as the interval between the first and last mounting allowed by females. Natural mating was used only once to serve females during the first 12 h after the beginning of estrus.

The percentage of females that ovulated was calculated as the total number of females ovulating / total of females treated x 100. The ovulatory rate was defined as the total number of *corpus luteum* per experimental group, divided by the total number of goats that ovulated within the experimental group. These two variables which previously mentioned were measured 10 d after the eCG administration by means of a transrectal echography. The diagnosis of pregnancy was performed by a transrectal echography at 45 d after the administration of eCG (Fig. 1).

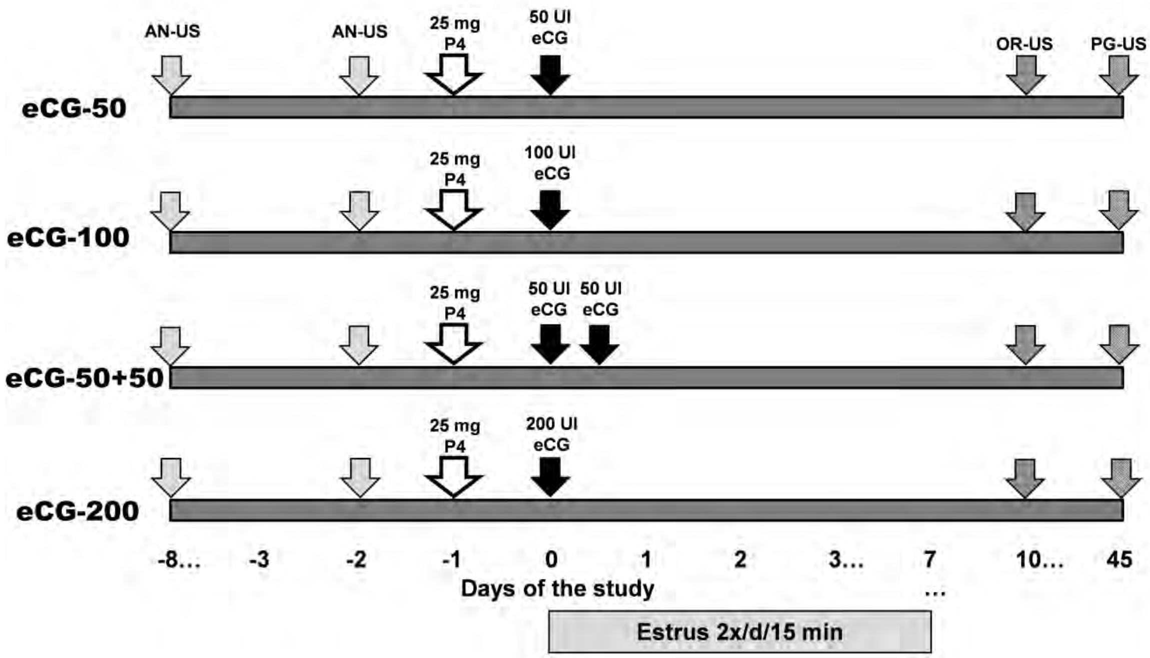

Figure 1. Schematic representation of the experimental protocols used to induce reproductive activity in Creole goats during the transition period (end of seasonal anoestrus, June). The goats were previously treated with intramuscular progesterone (P4), later, the experimental treatments, consisting in different doses (50, 100, 50+50 or 200 IU) of equine chorionic gonadotropin (eCG) were applied. The transrectal echography (US) was performed on days -8 and -2 in order to define the anovulatory (AN) state of the goats. At day 10 and 45, the ovulatory rate (OR) and pregnancy (PR) were confirmed, respectively.

Statistical analysis: The estrus latency, estrus duration and ovulatory rate were analyzed using an ANOVA and mean comparisons were performed by the Tukey Test, when required. The percentage of females in estrus, ovulation and pregnancy were analyzed using a contingency table and the chi-square test. All the statistical analyses were calculated using the statistical package SPSS 15.0. Significance level 0.05 was considered for all tests.

RESULTS AND DISCUSSION

On this study different doses of eCG were evaluated successfully induced estrus and ovulation in goats that were previously treated with intramuscular progesterone during seasonal transition reproductive period. The total percentage of females on estrus and ovulating in the experimental groups was 94.5% (37/39) without differences among experimental groups ($p>0.05$), (Table 1). The total percentage of time elapsed between the eCG administration and the estrus onset did not differ among the eCG-200, eCG-100 and eCG-50+50 groups (47.2 ± 10.86 h; $p>0.05$); yet, the largest value was observed in the eCG-50 group (94.86 ± 9.98 h; $p<0.05$). Besides, estrus induction occurred between 36 and 48 h after eCG administration in those experimental groups with doses equal or higher than 100 IU eCG (Fig. 2). All the goats receiving doses equal or higher than 100 IU showed estrus and 70% of the females were synchronized from 36 to 48 h post-eCG administration 100% of ovulation was achieved in all the treated goats. Our results are consistent with those reported in other studies, similar doses of eCG and hCG were used in anestrous goats previously treated with intramuscular progesterone (Alvarado-Espino *et al.*, 2016; Carrillo *et al.,* 2019). The high number of estrous goats could have been due to the ability of eCG to activate the LH and FSH receptors in both the theca and the granulosa cells, respectively (Murphy, 2012). Such physio-endocrine scenario possibility induced follicular growth, increased in estradiol secretion and subsequently the presence of the estrus activity (De-Rensis and López-Gatius, 2014).

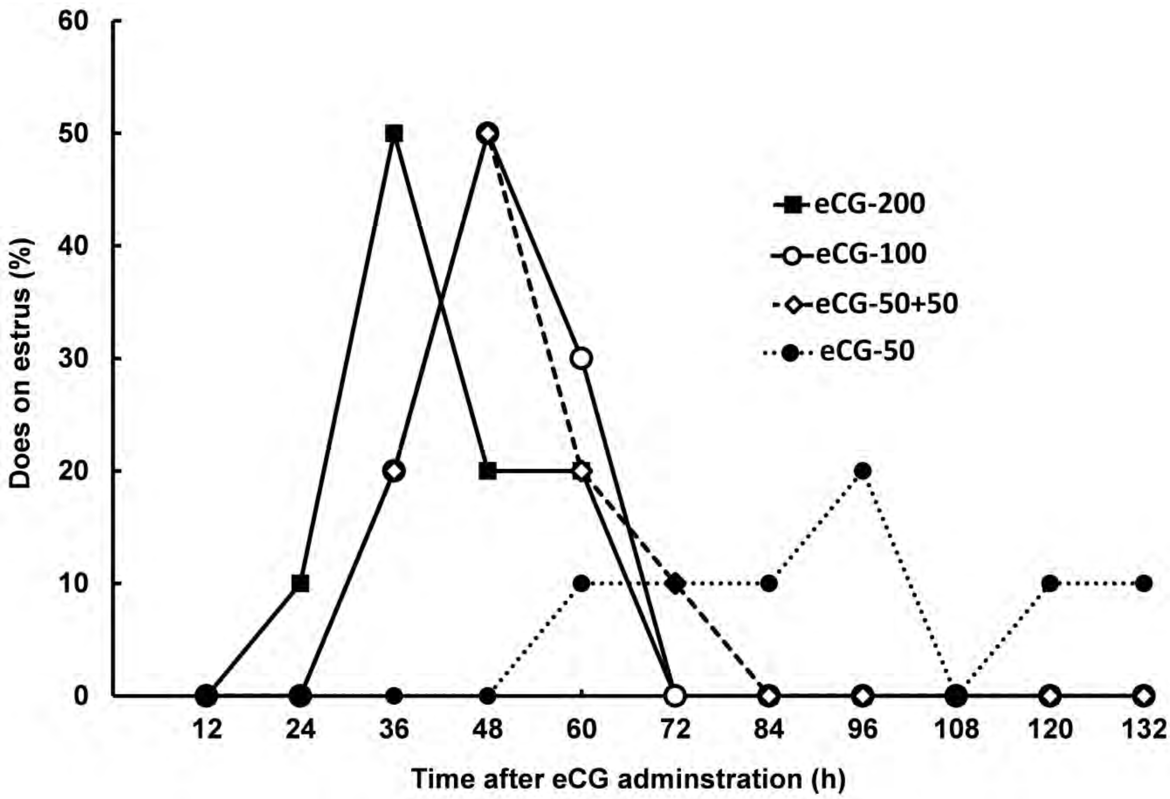

Figure 2. Time elapsed after the administration of the equine chorionic gonadotropin (eCG) until the appearance of estrus behavior in Creole goats managed under extensive conditions. All the females were treated at the end of the seasonal anestrus (June, 25ºN) with intramuscular progesterone and 24 h later, with different doses of eCG (200, 100, 50+50, 50 IU).

With respect to the high percentage of females ovulated it was observed that doses greater than 100 IU of eCG were enough to induce reproductive activity. Under such scenario, it is possible to suggest that eCG induced a positive feedback to estradiol, triggering the preovulatory LH suge, causing ovulation in most of the treated goats (Murphy, 2012; Carrillo *et al.,* 2019). It is worth mentioning that our result regarding goats ovulating are similar to those obtained with higher doses of eCG (300 IU; Vilariño *et al*., 2011). In fact, the high response to the low doses of eCG could have been promoted because this hormone has a relatively long-lasting bioactivity period, potentiating its effects on the ovarian activity, while inducing follicular growth and warranting ovulation (McIntosh *et al.*, 1975).

The total percentage of pregnant females for groups eCG-200, eCG-100 and eCG-50+50 was 73% (22/30; $p>0.05$). The lowest pregnancy rate (22%; 2/9; $p<0.05$) was recorded in the eCG-50 group (Table 1). In different studies, goats subjected to treatment protocols with progestogens + eCG, the pregnancy rate ranged from 61% (Martemucci and D'Alessandro, 2011) to 69% (Leboeuf *et al.,* 2003). In this study, no differences occurred among the experimental groups with doses equal or higher than 100 IU of eCG. Observing an important percentage of pregnant goats (73%), which confirms the interesting reproductive response obtained in goats treated with eCG. This could be explained because of the effect that eCG exerts upon both the theca and the granulosa cells, giving place to a bigger preovulatory follicle growth, as well as due to the eCG´s luteotrophic effect, which favors the *corpus luteum* growth and functionality (Thatcher *et al*., 2001). In fact, a *corpus luteum* with a bigger diameter increases its progesterone secretion (García-Pintos and Menchaca, 2016), favoring embryo implantation and the subsequent pregnancy.

The estrus and ovulatory responses of the goats treated with 50 IU of eCG was acceptable (78%) nonetheless, the dose of eCG and the asynchrony of the estrus activity could negatively influence the percentage of pregnant goats (22%). Possibly, this eCG dose was unable to reach the optimum threshold to correctly perform its different functions, such as a synchronized follicular growth, ovulation, and luteinization of the *corpus hemorrhagicum*. Indeed, a poor luteinization of the follicular cells could negatively affect the growth of the *corpus luteum* and, consequently, an abnormal progesterone synthesis.

Table 1. Reproductive outcomes in Creole goats during the seasonal anestrus (transition period, during June) treated with either 200 (eCG-200), 100 (eCG-100), 50+50 (eCG-50+50) and 50 (eCG-50) IU of eCG. Doses were administered 24 h after the administration of 25 mg of intramuscular progesterone

	Treatment group			
Variables	**eCG-200**	**eCG-100**	**eCG-50+50**	**eCG-50**
Goats in estrus (%)	10/10 (100)	10/10 (100)	10/10 (100)	7/9 (78)
Latency to estrus (h)	42 ± 3.69^{b}	49.2 ± 2.8^{b}	50.4 ± 3.49^{b}	94.86 ± 9.98^{a}
Duration of estrus (h)	32.4 ± 5.38	34.8 ± 4.18	39.6 ± 5.67	34.29 ± 9.23
Goats ovulating (%)	10/10 (100)	10/10 (100)	10/10 (100)	7/9 (78)
Ovulation rate (n, CL)	1.4 ± 0.22	1.5 ± 0.27	1.6 ± 0.22	1.43 ± 0.20
Goats pregnant (%)	$7/10^{a}$ (70)	$7/10^{a}$ (70)	$8/10^{a}$ (80)	$2/9^{b}$ (22)

^{a,b,} Means with different superscripts within rows differ (P<0.05).

Thus, dysfunctions of the *corpus luteum* promote embryonic losses and diminish pregnancy rate (Samir *et al.,* 2016). It would be convenient to perform more studies with these hormonal protocols and the intramuscular administration of progesterone plus eCG or hCG in different breeds and management systems as well as different periods of the seasonal anestrus, in order to be able to respond to these questions.

CONCLUSSION

With the evidence provided by this study, we can conclude that the administration of intramuscular progesterone plus doses greater than 100 IU of eCG are effective hormonal strategies to inducing sexual activity of Creole goats, under subtropical extensive production systems during the transitional anestrous period (25ºN, June). The protocol described here makes the use of exogenous hormones more efficient, with reduce doses, decreasing expenses and is practical use by goat producers. This results can will apply to other ruminant industries.

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CONCLUSIONES GENERALES

ESTUDIO 1

Este estudio revela el efecto del rango social sobre la inducción de la actividad reproductiva en cabras anéstricas, sometidas a un protocolo hormonal con progesterona intramuscular más eCG (100 o 350 UI). La mayoría de las variables de reproductivas se vieron afectadas por el rango social (porcentaje de cabras en estro, duración del estro, porcentaje de cabras que ovularon, tasa ovulatoria y en el tamaño del cuerpo lúteo), favoreciendo a las cabras de alto rango. Además, la tasa ovulatoria y el tamaño de camada aumentó, mientras que la latencia al estro disminuyó en el grupo HSR+D350. Las cabras de menor clasificación fueron menos pesadas en comparación con los otros dos grupos sociales, lo que indica una fuerte competencia por el alimento. Dado que no hubo diferencias entre las dosis de eCG con respecto a las variables más importantes de la respuesta reproductiva, se concluye en disminuir la cantidad de hormonas exógenas. La disminución de hormonas exógenas para el control reproductivo de los animales domésticos, encuadra con las reglamentaciones vigentes del bienestar animal y la seguridad alimentaria. Estos hallazgos son relevantes no solo desde el punto de vista conductual, fisiológico y del bienestar animal, sino que también, adquieren un significado importante en la eficiencia productiva de los caprinos.

ESTUDIO 2

Con las evidencias que proporciona este estudio, podemos concluir que una aplicación de progesterona intramuscular más 100 UI de eCG, pude inducir efectivamente la actividad sexual en cabras criollas del subtrópico mexicano durante el periodo de transición (25°N, junio). Además, el protocolo aquí descrito hace eficiente el uso de hormonas exógenas, disminuye los costos y es práctico para el uso de los productores.