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EVALUACION DE LOS FACTORES QUE INFLUYEN SOBRE LA TASA DE PREÑEZ Y LA SUPERVIVENCIA EMBRIONARIA EN RECEPTORAS DE EMBRIONES BOVINOS PRODUCIDOS IN-VITRO

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A Dios que es el pilar de mi vida y todos mis proyectos.
A mi madre que me ha acompañado y apoyado siempre.
A mis profesores que me enseñaron y guiaron en todo este proceso de aprendizaje.

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Resumen

EVALUACION DE LOS FACTORES QUE INFLUYEN SOBRE LA TASA DE PREÑEZ Y LA SUPERVIVENCIA EMBRIONARIA EN RECEPTORAS DE EMBRIONES BOVINOS PRODUCIDOS IN-VITRO

La producción de embriones *in vitro* (PIV), en los últimos años se ha convertido en una biotecnología reproductiva de importante uso en los sistemas productivos bovinos. Sin embargo, por sus costos, es poco asequible para los pequeños y medianos productores. Aunque existen algunos criterios específicos para la selección de hembras aptas para ser receptoras de embriones, es necesario identificar los factores que pueden afectar negativamente el éxito de los programas masivos de transferencia de embriones PIV. El objetivo de este estudio fue evaluar los factores asociados con la tasa de preñez y la supervivencia embrionaria en receptoras bovinas en un programa de transferencia de embriones producidos *in vitro* en el Departamento de Cundinamarca. Se realizaron n=4083 transferencias de embriones, en 26 municipios del departamento de Cundinamarca. En las receptoras se evaluaron, factores intrínsecos y extrínsecos que podían influir sobre la probabilidad de preñez. Además, se evaluaron algunos factores medioambientales como temperatura corporal y medioambiental al momento de la trasferencia, y el nivel de exposición a patógenos que afectan la reproducción a través de la realización de pruebas serológicas para: *Diarrea Viral Bovina, Herpes Virus Bovino-1 , Brucella abortus, Leucosis Bovina, Neospora caninum, y seis serovares de Leptospira (L. Hardjo prajinto, L. Hardjo bovis, L.pomona, L. canicola, L. grippotyphosa L. Icterohaemorrhagiae*). Se tuvieron en cuenta como factores de exclusión, animales persistentemente infectados de DVB (PI-

DVB), positivos a *B. abortus* y a *N. caninum.* Adicionalmente, se evaluó el estado de salud de las receptoras a través de la realización de pruebas de función hepática, renal, hemograma y de parásitos gastrointestinales y su relación con el resultado del diagnóstico de gestación y la supervivencia embrionaria. Los diagnósticos de gestación y de viabilidad gestacional se realizaron el día 30 y 60 respectivamente, post-transferencia. Los datos fueron analizados con un modelo de regresión logística binaria. La TP promedio del estudio fue del 26,1%. Los factores asociados a la probabilidad de preñez viable fueron la vacunación contra enfermedades reproductivas (OR= 0.4, IC 95% (0.2-0.6), p= 0.001), uso de semen convencional en la FIV de los embriones PIV (OR= 0.6 , IC 95% 0,4-0,9, p = 0,009), la adecuada ciclicidad ovárica de las receptoras al momento de la sincronización del estro (OR=1,2, IC 95% 1. 1 – 1.5, p=0.025), volumen del cuerpo CL al momento de la ET (OR = 1,9, IC del 95 %: 1,0-3,8, p=0,05), temperatura ambiental (OR= 1,6, IC 95% 1,2-2,2 P=0,005), Humedad Relativa (OR = 1.9, IC 95% 1.3-2.7, p = 0.001). La pérdida gestacional del estudio fue de 8.3%, asociada a los factores de exposición a HVB-1(OR= 5,4, IC 95% 1,1-27,6, p = 0,043), *L. hardjo bovis* y *L canicola* (OR= 4,0, IC 95% 1,1-14,9, p=0,032) y (OR= 8,9, IC 95% 1,6-14,0, p=0,021), respectivamente, cambios en la condición corporal (OR= 3,6, IC 95% 1,1-12,8, p =0,048), temperatura corporal (OR = 0,1, IC 95 % 0,007-0,8, p = 0,03) e Índice de Temperatura/Humedad (OR= 0,023, IC 95% 0,001-0,775, p =0,036). Los resultados de esta investigación son importante evidencia para la estructuración de programas de transferencia de embriones, en cuanto a las consideraciones para la selección de receptoras, estatus sanitario de las mismas, factores ambientales, factores intrínsecos/extrínsecos, en pro de la tasa de preñez y la supervivencia embrionaria.

Palabras clave: Embriones bovinos, embriones in-vitro, tasa de preñez, enfermedades infecciosas reproductivas

Abstract

EVALUATION OF THE FACTORS THAT INFLUENCE THE PREGNANCY RATE AND EMBRYO SURVIVAL IN RECIPIENTS OF BOVINE EMBRYOS PRODUCED IN-VITRO

In recent years the *in vitro* embryos production (PIV), has become into one of the most important reproductive biotechnologies for the bovine reproductive system. Nevertheless, for its cost, it's not affordable for small and medium producers. Though there are some criteria to select the suitable female for being the embryos receiver, it's necessary to identify the factors could affect in a negative way the success of PIV embryos transfer massive programs. This study goal was to evaluate the factors associated to the pregnancy rate and embryo survival in bovine receptors in an in vitro produced embryos transfer program in the Department of Cundinamarca. N=4083 embryos transfers were made in 26 municipalities of the department of Cundinamarca. In the receptors was evaluated intrinsic and extrinsic factors that may influence the pregnancy probability. Further some environmental factors were evaluated, like body and environmental temperature at the time of transfer, and the level of exposure to pathogens that affects the reproduction through serological tests to: *Bovine Viral Diarrhea, Bovine Herpes Virus-1, Brucella abortus, Bovine Leukosis, Neospora caninum, and six serovars of Leptospira (L. hardjo prajinto, L. hardjo bovis, L. pomona, L. canicola, L. grippotyphosa L. Icterohaemorrhagiae)*. They were considered as exclusion factors, animals persistently infected with BVD (PI-BVD), positive for *B. abortus* and *N. caninum*. Besides the health status of the receptors was evaluated through the liver, kidney function, blood count and gastrointestinal parasites test and their

relationship with the result of pregnancy diagnosis and embryonic survival. Pregnancy and gestational viability diagnoses were performed post-transfer on the 30th and 60th day. The data was analyzed with a binary logistic regression model. The average pregnancy rate in the study was 26.1%. The factors associated to the probability of viable pregnancy were vaccination against reproductive diseases (OR= 0.4, IC 95% (0.2-0.6), p= 0.001), use of conventional semen in IVF of the embryos PIV (OR= 0.6, IC 95% 0,4-0,9, p = 0,009), the adequate ovarian cyclicity of the receptors at the time of estrous synchronization (OR=1,2, IC 95% 1. 1 – 1.5, p=0.025), CL body volume at time of ET (OR = 1,9, IC del 95 %: 1,0-3,8, p=0,05), environmental temperature (OR= 1,6, IC 95% 1,2-2,2 P=0,005), relative humidity (OR = 1.9, IC 95% 1.3-2.7, p = 0.001). The study gestational loss was 8.3%, associated with exposure factors to HVB-1 (OR= 5,4, IC 95% 1,1-27,6, p = 0,043), *L. hardjo bovis and L. canicola* (OR= 4.0, 95% CI 1.1-14.9, p=0.032) and (OR= 8.9, 95% CI 1.6-14.0, p= 0.021), respectively, changes in body condition (OR= 3,6, IC 95% 1,1-12,8, p =0,048), body temperature (OR = 0,1, IC 95 % 0,007-0,8, p = 0,03) e Temperature/Humidity index (OR= 0,023, IC 95% 0,001-0,775, p =0,036). The results of this research are essential evidence for the structuring of embryo transfer programs in terms of considerations for the selection of recipients, their health status, environmental factors, and intrinsic/extrinsic factors in favour of the pregnancy rate and the survival of the embryo.

Keywords: Bovine embryos, in-vitro embryos, pregnancy rate, reproductive infectious diseases.

Contenido

Introducción

El uso de biotecnologías reproductivas en los sistemas pecuarios tiene como fin primordial el mejoramiento productivo y reproductivo de las ganaderías. Hoy por hoy la producción de embriones in vitro (PIV), ofrece la posibilidad de maximizar el potencial genético de la hembra bovina en pro de la productividad del sistema. La fertilización in vitro (FIV), en los últimos años ha venido en ascenso en los sistemas productivos bovinos en Colombia, sin embargo, por sus costos, es poco asequible para los pequeños y medianos productores. Cundinamarca es por naturaleza un departamento agropecuario, con un gran potencial económico dentro de la economía Colombiana, sin embargo su valor productivo se ha visto afectado por el comportamiento del sector a nivel nacional, el impacto de las marcadas épocas climáticas, atraso de la aplicación de tecnologías que le ayuden al mediano y pequeño productor a ser más productivo y competitivo, lo cual desfavorece el aprovechamiento de los recursos y capacidades de los municipios del departamento impidiendo la tecnificación y especialización productiva (Gob. Cundinamarca, 2016). La producción de embriones en Colombia es una herramienta usada en beneficio del mejoramiento genético y la competitividad de las producciones ganaderas a través de animales con mayor potencial de producción, sin embargo existe la necesidad de incrementar la eficiencia de los programas PIV en términos de optimizar los resultados y reducir efecto negativo de diversos factores sobre la tasa de preñez y supervivencia embrionaria.

En el año 2020 The International Embryo Technology Society (IETS), reportó un incremento de la producción de embriones PIV en un 12,1% y del 10,2% en la transferencia de embriones (ET). La producción total mundial de PIV y ET para este año fue de 1’156.442 y 878.181 respectivamente; Norte América produjo 578.995 embriones y 339.716 transferencias; de igual manera Sur América tuvo una producción 500.397 y 474.145 de embriones PIV y ET respectivamente (IETS, 2020). En Colombia el uso de embriones PIV para el mejoramiento genético bovino se ha incrementado de gran manera en los últimos 10 años. Sin embargo se cuenta con pocos datos que reporten el éxito de programas de transferencia de embriones PIV en Colombia (Oyuela, L.A.& Jimenez, 2010). Recientemente se publicaron datos de viabilidad y tasa de preñez (TP) de producción de embriones a gran escala, reportando una TP de 46% (Bonilla León, Mejía Gallego, Gómez Domínguez, Torres Londoño, & Uribe García, 2018). En el 2009, Oyuela reportó una TP para embriones PIV del 38% (462/1227) en un estudio realizado en receptoras cebú Brahman gris, rojo y Gyr (Oyuela L., 2009). Farfán en el 2005 reportó una TP de 37,1% (113/298) en un estudio realizado en el Municipio de Ventaquemada/Boyacá, con hembras receptoras de embriones de raza Holstein, Normando y Brangus (Farfan Rojas & Porras Vargas, 2013). Recientemente se realizó un estudio en Montería-Córdoba en ganaderías doble propósito usando receptoras mestizas F1 cebú x Bos Taurus y se reportó que las novillas tuvieron una TP del 39% (39/100) mientras que en vacas la TP fue del 17,8% (7/39). La variabilidad del resultado de estos programas expresado en función de la TP puede estar estrechamente influenciado por diferentes factores extrínsecos, intrínsecos o medioambientales, tales como los incluidos en el presente estudio.

Diferentes investigaciones han demostrado que el éxito de los programas de transferencia de embriones PIV, han sido influenciados por diversos factores, los cuales se pueden entender como factores intrínsecos, siendo estos los relacionados directamente con la receptora de los embriones, su estatus sanitario, exposición a enfermedades que afectan la reproducción, calidad de cuerpo lúteo (CL), entre otros; factores extrínsecos, tales como el medio ambiente o aquellos directamente relacionados con el embrión, uso de la técnica de TE e incluso la persona quien la realiza. Autores como Roper et al, realizaron un estudio en donde evaluaron el impacto de diferentes factores sobre la TP en n=256 hembras

receptoras de embriones y evidenciaron relación directa del momento de la TE, tercio del cuerno donde se depositó el embrión (P=0,08), tiempo de duración de la TE (P=0,03) y grado de dificultad presentado durante la TE (P=0,07) con la TP (Roper et al., 2018). Abdel Aziz et al., reporto la influencia del grado de calidad del embrión transferido sobre la TP, siendo que embriones de calidad 1 (excelente) y calidad 2 (bueno) tuvieron mejor TP (P<0,05), 79,17% y 65,38% respectivamente (Abdel Aziz, Khalil, & Bard, 2022). De igual manera otro estudio encontró que la calidad del embrión transferido (P=0,01) influyo significativamente en la TP de n=651 novillas preñadas por transferencia de embriones (Chebel, Demetrio, & Metzger, 2008). Oyuela et al, en un estudio realizado en tres centrales de receptoras de embriones, en donde se evaluaron factores bajo la categorización de factores extrínsecos e intrínsecos, reporto que la dificultad al momento de la TE (p<0,001, OR 5,071 IC 95%), la calidad del embrión transferido (p=0,001, OR 2,280 IC 95%), el tiempo trascurrido desde la salida del embrión del laboratorio hasta el momento de la TE (p<0,05 , OR 1,434 IC 95%), influyeron significativamente sobre la TP de las receptoras de embriones PIV (Lino Andres Oyuela, 2009).

La exposición a enfermedades infecciosas reproductivas puede ser un factor influyente sobre la TP en receptoras de embriones PIV, siendo el virus de Diarrea Viral Bovina (VDVB), Herpes Virus Bovino Tipo 1 (HVB-1), N. caninum, Leucosis Bovina Enzootica (VLB) y Leptospira las principales enfermedades involucradas en problemas reproductivos en los hatos ganaderos. Un estudio realizado en Brasil en el año 2016 en un programa de transferencia de embriones PIV reportó que el 74.04% (174/235) de las receptoras presentaron títulos a DVB, logrando una TP del 77% (42/54), sin embargo el 81,82% (9/11) de las receptoras gestantes que presentaron títulos a DVB presentaron aborto (P <0.05), de igual manera reporto que el 43,82% (103/132) de las receptoras presentaron anticuerpos contra HVB-1, adicionalmente reportaron una baja TP 28,5% (20/70), sugiriendo una circulación activa del virus, para *N. caninum* reporto que de 235 receptoras, el 63,82% (150/235) fueron positivas, con una TP de 34% (32/93) y 5 abortos y del total de receptoras sincronizadas, el 54,47% (128/235) tenían títulos que indicaban ser positivas a Leptospira, se presentaron 11 abortos, de los cuales 3 correspondieron a hembras seropositivas, una tenía títulos contra *L. hardjo* (1:600) y dos tenían títulos (1:200) contra *L. icterrohaemorragiae.* Para este estudio al autor reporta una TP de 34.2% (54/158), y una perdida gestacional de 20,37% (11/54), las cuales ocurrieron entre los 35 a 75 días de

gestación, concluyendo que este periodo comprende el desarrollo primario de la gestación, la cual es muy susceptible a daños asociados a la presencia de agentes infecciosos, lo cual coincide con los resultados obtenidos (Diniz et al., 2016). En Colombia, un estudio realizado en 840 novillas destinadas a ser receptoras de embriones ubicadas en Puerto Boyacá encontró que el 25,83% (217/840) de las receptoras era seropositiva a DVB, el 19,29% (162/840) a Neospora y el 14,64% (123/840) eran seropositivas a LVB, lo cual según el autor evidencio la presencia de enfermedades infecciones reproductivas en las receptoras de embriones, lo cual responde a falencias en el manejo sanitario de los hatos y puede repercutir en el desempeño reproductivo de las receptoras (Naranjo Guerrero, Rodríguez Colorado, & Mejía Araque, 2022).

La transferencia de embriones PIV representa una de las alternativas de mejoramiento genético más importantes en la actualidad, en búsqueda de alcanzar un mejor rendimiento productivo y reproductivo en las ganaderías, sin embargo el éxito de estos programas de transferencia de embriones PIV se puede ver significativamente afectado por la influencia de diferentes factores como los ya mencionados, además se conocen pocos reportes sobre el impacto que puede tener la exposición a enfermedades infeccionas reproductivas sobre la TP y la supervivencia embrionaria en programas de transferencias de embriones PIV, lo cual repercute económicamente en la implementación de estos programas. El objetivo de este estudio fue evaluar la exposición a patógenos que afectan la reproducción bovina, la influencia de diferentes factores, sobre la TP y la supervivencia embrionaria en un programa de transferencia de embriones PIV en el departamento de Cundinamarca.

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1. Factores que influyen sobre la probabilidad de preñez y supervivencia embrionaria en receptoras bovinas de embriones producidos in-vitro. Revisión Bibliográfica

Resumen

El uso de Técnicas de Reproducción Animal Asistida (TRA) ha cobrado importancia en los sistemas ganaderos, siendo la transferencia de embriones producidos *in-vitro* una de las más implementadas en la actualidad. La eficiencia de la transferencia de embriones producidos *in-vitro*, en relación con la tasa de preñez y la supervivencia embrionaria se puede ver afectada por la influencia de diversos factores y la exposición a enfermedades infecciosas que afectan la reproducción de las hembras bovinas usadas como receptoras de embriones. La presente revisión aborda información relacionada con las enfermedades infecciosas que afectan la reproducción de la hembra bovina y los factores que influyen sobre la tasa de preñez en programas de transferencia de embriones.

Palabras clave: transferencia de embriones, producción de embriones in-vitro, tasa de preñez, supervivencia embrionaria.

Introducción

La transferencia de embriones bovinos *in-vitro* (PIV) es una de las técnicas de Reproducción Animal Asistida (TRA) que se usan en la actualidad ganadera para el mejoramiento genético de los animales en pro de la productividad y rentabilidad de las explotaciones pecuarias, siendo una de las biotecnologías más usadas en la actualidad. La implementación de estos programas de transferencia de embriones (TE/PIV) ha tenido un auge comercial en las explotaciones ganaderas, sin embargo, se tiende a ignorar el impacto que pueden tener diferentes factores sobre el éxito de la TE/PIV reflejado en la tasa de preñez (TP) y la supervivencia embrionaria. Se ha descrito que tanto enfermedades infecciosas reproductivas como factores específicos extrínsecos e intrínsecos pueden tener una influencia sobre la TP de embriones PIV.

Se han realizado diferentes investigaciones sobre el efecto de algunos factores sobre la TP en receptoras de embriones tanto producidos *in-vitro* como *in-vivo.* Un estudio realizado en 561 receptoras de embriones reporto una relación positiva del diámetro del cuerpo lúteo con la TP, siendo que aquellas receptoras que tenían un CL de mayor diámetro tuvieron mayor TP ($P<0.05$). El valor promedio del diámetro del CL fue de 2,11 ± 0,46 cm en hembras gestantes y de 1,96 ± 0,34 cm en hembras no gestantes ($P< 0,05$) (Alkan et al., 2020). Otro estudio realizado en un programa de TE/PIV encontró que las receptoras con CL de mayor diámetro (19.54 ± 1.33 mm) tuvieron mayor TP ($P<0.05$), además también describió que las receptoras con CL en el ovario izquierdo tuvieron mayor TP 42% (9/ 21) que las receptoras que tenían el CL en el ovario derecho 13,8% (4/29) ($p=0.0275$) (Pelagio et al., 2022).

Un estudio retrospectivo basado en información obtenida de granjas lecheras de Florida, USA, reportaron el efecto del estado de desarrollo del embrión ($P < 0.001$), tipo de embrión

(in-vivo/in-vitro, fresco/congelado) (P < 0.001), calidad del embrión (P < 0.001), índice de temperatura y humedad al momento de la TE (P= 0.02); la TP fue mayor en receptoras que recibieron embriones frescos (49,3%), blastocistos expandidos (45.3%), embriones de excelente calidad (42.2%) y cuando el THI fue <72 (39.7%) (Ferraz et al., 2016). Otro estudio realizado en un programa de transferencia de embriones producidos *in-vivo*, reporto una TP de 32,17% además de que los embriones depositados en el tercio medio del cuerno uterino tuvieron mejor TP (48,8%) (P<0.05) (Ongubo et al., 2015). Un estudio reporto una TP del 55.0% en un programa de TE/in-vivo, los factores correlacionados con la TP fueron la calidad del embrión (P < 0.01), tipo de embrión (fresco/congelado) (P < 0.01), época del año (verano/invierno) (P = 0.04) y el técnico que realizo la TE (P < 0.01) (Vandaele, 2010). Oyuela et al, en un estudio realizado en tres centrales de receptoras de embriones, reporto que la dificultad al momento de la TE (p<0,001, OR 5,071 IC 95%), la calidad del embrión transferido (p=0,001, OR 2,280 IC 95%), el tiempo trascurrido desde la salida del embrión del laboratorio hasta el momento de la TE (p<0,05 , OR 1,434 IC 95%), influyeron significativamente sobre la TP de las receptoras de embriones PIV (Lino Andres Oyuela, 2009).

La eficiencia reproductiva es uno de los aspectos más relevantes en los sistemas de producción bovina. Los problemas reproductivos que se presentan en las ganaderías en gran parte son generados por la presencia de agentes infecciosos, que comprometen la salud reproductiva de los animales y la viabilidad del embrión y feto, esto tiene una importante implicación en el éxito reproductivo de los sistemas productivos y la implementación de biotecnologías reproductivas (Diniz et al., 2016; Yoo, 2010). En programas de transferencia de embriones PIV se tiene poca información de la relevancia de estas enfermedades reproductivas infecciosas. Un estudio realizado en Brasil realizo un estudio de diagnóstico serológico de enfermedades infecciosas (brucelosis, leptospirosis, DVB, IBR, neosporosis) en 235 receptoras de embriones en un programa de transferencia de embriones PIV. Las enfermedades de más alta presentación, de acuerdo con el diagnóstico serológico fueron DVB (74.04%) y Neosporosis (63.83%). Adicionalmente se reportó que al menos el 36.17% de las receptoras eran positivas a 3 enfermedades y el 33.19% a dos enfermedades. La presentación de abortos estaba relacionada con la presencia de al menos una enfermedad (P < 0.05). El porcentaje de abortos fue del 20.37% (11/54), según la seropositividad de las receptoras la relación de

la presentación de abortos con DVB fue de 81.82% (9/11), 72.73% (8/11) con IBR, 45.45% (5/11) con Neosporosis, 27.27% (3/11) con Leptospirosis y 18.18% (2/11) con Brucelosis. La industria ganadera y los programas de biotecnología podrían verse afectados por los efectos negativos de estas enfermedades, por lo cual se hace necesario tener en consideración el estado de salud de los animales con el fin de reducir las pérdidas en los programas de TE (Diniz et al., 2016).

Este articulo tiene la intención de hacer una revisión y recopilación de información, datos y evidencia de la asociación y el efecto que pueden tener diversos factores tanto extrínsecos como intrínsecos sobre la tasa de preñez en programas de transferencia de embriones producidos *in-vitro*, además de establecer la premisa del efecto que pueden tener las enfermedades infecciosas reproductivas en el éxito de la transferencia de embriones bovinos en relación con la tasa de preñez y/o perdidas embrionarias.

FACTORES QUE INFLUYEN SOBRE LA TASA DE PREÑEZ Y LA SUPERVIVENCIA EMBRIONARIA

Factores Extrínsecos

Medioambientales

La fertilidad se puede ver deteriorada por efecto de algunos factores, que se ven reflejados en procesos fisiológicos como, la reducida expresión de celo, alteraciones en la fisiología ovárica y muerte embrionaria temprana (Negrón-Pérez, Fausnacht, & Rhoads, 2019). Uno de los factores que más afecta la tasa de preñez es el estrés calórico (Oyuela & Jimenez, 2010). Cuando la exposición al estrés calórico es constante, los animales tienden a disminuir el consumo de alimento, esto genera una reducción de la tasa metabólica y modifica el funcionamiento hepático, esto se ve reflejado en procesos fisiológicos reproductivos como baja presentación de celo, ciclicidad ovárica reducida, bajas concentraciones de hormonas esteroideas, alteraciones morfológicas de los oocitos, alteraciones y muerte embrionaria temprana (Negrón-Pérez et al., 2019); lo cual perjudica significativamente la producción, reproducción y aumenta la sensibilidad a enfermedades

infecciosas (Nardone, Ronchi, Lacetera, Ranieri, & Bernabucci, 2010). El estrés calórico también se ha asociado con el deterioro del desarrollo del embrión y el aumento de la mortalidad embrionaria en los bovinos, partiendo de que los embriones en su estado de desarrollo temprano son altamente termo sensibles (Hansen, 2007).

La eficiencia productiva de las ganaderías puede verse afectada por factores ambientales como temperatura ambiental, humedad relativa, precipitaciones. Las demarcadas variaciones medioambientales repercuten en la producción y reproducción de los animales (Julon, Burga, Bardales, & Puicón, 2018). Las altas temperaturas retrasan el desarrollo embrionario, porque los embriones tienen una menor capacidad de producción de IFNT-t el cual es necesario para el reconocimiento materno de la gestación (Julon, Burga, Bardales, & Puicón, 2018). Algunos estudios reportan el efecto del Índice de Temperatura y Humedad (THI), sobre la tasa de preñez. Un estudio realizado en el 2016 reporto la TP de novillas y vacas receptoras de embriones PIV, con un THI<72 y THI 72-70, THI>72 (41.5%, 43.9%, 37.0% respectivamente), siendo que las receptoras que se encontraron con un THI<72 tuvieron una mayor TP ($P < 0.02$). No encontraron diferencia significativa ($P=0.25$) entre la TP de las receptoras con THI<72 y THI 72-70. (Ferraz et al., 2016); según estos resultados los autores indicaron que las novillas pueden tener mejor capacidad de regular la temperatura corporal en condiciones de estrés calórico (Sartori et al., 2010). Un estudio realizado en Rio Branco, Brasil reporto que el aumento del THI redujo la TP en receptoras de embriones Nelore PIV. La TP más baja (33,3%) se reportó cuando el THI fue de 75,7, respecto a la TP más alta (56,3%) cuando el THI fue de 75,1, concluyendo que el aumento de la THI reduce la TP (Cordeiro et al., 2020). Colombia por ser un país tropical sus épocas climáticas están demarcadas por la lluvia y la sequía, sin embargo no se ha encontrado que estas épocas climáticas afecten negativamente la TP (Oyuela L., 2009).

Condición Corporal (CC)

Un adecuado manejo nutricional en las hembras receptoras de embriones, establece un equilibrio de hormonas metabólicas, dentro de las cuales se destaca la Leptina, el Factor de Crecimiento Insulínico Tipo I (IGF-1) y Ghrelina, las cuales a través de su acción metabólica influyen en la endocrinología del sistema reproductivo (Occhio, Baruselli, & Campanile, 2018). La evaluación de la CC está relacionada con el nivel de reservas corporales de las que puede disponer un animal para suplir los requerimientos de mantenimiento de funciones fisiológicas y de producción (Vasquez Ch. & Amilcar Bò., 2017). Un estado de malnutrición y baja CC tiene un importante efecto negativo sobre los procesos fisiológicos reproductivos; la deficiencia energética repercute sobre la liberación de GnRH y pulsos de LH en hembras bovinas (Guerra & Álvaro, 2004; Vasquez Ch. & Amilcar Bò., 2017). Santos et al, en un programa de IA de 6936 vacas, reporto que vacas que no tuvieron cambios significativos en el puntaje de condición corporal (BCS), tuvieron 1,7 veces más probabilidades de concebir que aquellas que perdieron uno o más unidades en el BCS. El BCS también influyó en la pérdida gestacional, ya que vacas que no tuvieron cambio en el BCS o perdieron menos de una unidad, tuvieron menor riesgo de pérdida gestacional al día 60 ($P<0,05$) (Santos, Rutigliano, & Sà Filho, 2009). Un estudio realizado en Lima, Perú en receptoras de embriones PIV, reporto baja TP en receptoras cuya CC era baja ($CC<2.5/5$) ($P=0.022$) reportando una TP del 26%, sugiriendo que las receptoras con baja CC tienen una alta incidencia de regresión del CL con la consiguiente pérdida gestacional (Pelagio et al., 2022).

Exposición a enfermedades infecciosas reproductivas

En la actualidad se cuentan con diversos reportes sobre la presencia de enfermedades infecciosas que afectan la reproducción y se conoce que estas enfermedades tienen importantes repercusiones sobre la salud reproductiva, especialmente de la hembra bovina.

En receptoras de embriones hay evidencia de un estudio realizado en 235 receptoras de embriones PIV evaluó la seroprevalencia de enfermedades infecciosas reproductivas en las receptoras, el 2,89% (7/235) fueron seropositivas a *B. abortus*, adicionalmente el 18,18% de los abortos presentados fueron de receptoras seropositivas a *B. abortus*. Este estudio reporto una TP 67,23% (158/235), una perdida gestacional a los 30 y 75 días de preñez de 31,18% (54/158) y 20,37% (11/54) respectivamente, adicionalmente también se reportó seroprevalencia de 54,47% (128/235), 43,83% (103/253), 74,04% (174/235) y 63,82% (150/235) para Leptospira, HVB-1, DVB y *N. caninum* respectivamente. Adicionalmente los autores señalan que al menos el 8,5% de las receptoras fueron positivas a 4 enfermedades y el 36,17% y 33,19% de las receptoras fueron seropositivas a 2 y 3 enfermedades respectivamente. Del total de abortos el 45,4% fueron de receptoras seropositivas a 3 enfermedades y el 36,36% de receptoras seropositivas a 4 enfermedades (Diniz et al., 2016). Otro estudio realizado en una central de transferencia de embriones (in-vivo) en Brasil, evaluó la seropositividad de a *N. caninum* de 101 receptoras, 60 donadoras y 90 terneros, se reportó una prevalencia de general de *N. caninum* de 32.67%, el 32.67% de las receptoras, el 26.22% de las donadoras y el 6.66% de los terneros fueron positivos a *N. caninum* (de Oliveira, Álvarez-Garcia, Ortega-Mora, Borges, & da Silva, 2010). Un estudio realizado en Nueva Zelanda, reportó un aumento del riesgo de pérdida fetal en los animales seropositivos de *L. hardjo* (OR = 1.84; 95% IC = 1.01–3.33) y *L. pomona* en vacas no vacunadas (OR = 14.91, IC 95% = 1.73–128.84) (Sanhueza, Heuer, & West, 2013). De igual manera un estudio realizado en el Reino Unido, en 1051 vacas, reportó que la prevalencia de *N. caninum* fue mayor (18%) en vacas que habían abortado, mientras que la prevalencia de vacas sin aborto fue menor (6%) ($P<0.0001$). Lo cual es un indicativo de la gran relevancia que tiene la presencia de agentes infecciosos reproductivos los programas de TE y la repercusión que tiene sobre la incidencia de aborto o baja TP. A pesar de que se tiene importante evidencia del impacto de enfermedades infecciosas reproductivas sobre la eficiencia reproductiva de los hatos bovinos, se tiene poca evidencia especialmente en Colombia, de la influencia de estas enfermedades reproductivas en programas de transferencia de embriones in-vitro.

Factores Intrínsecos

Volumen del Cuerpo Lúteo

El Cuerpo Lúteo (CL) es un órgano endocrino transitorio primordial en la gestación, ya que es la principal fuente de Progesterona sérica (P_4), hormona de vital importancia para el reconocimiento, establecimiento y mantenimiento de la preñez en el bovino (Binelli, Thatcher, Mattos, & Baruselli, 2001). La relación del volumen del CL con la concentración de P_4, ha sido uno de los factores más estudiados en relación con la tasa de preñez en diferentes investigaciones, en donde se ha asociado que la concentración de P_4 es proporcional al tamaño del CL y de esta manera la concentración de P_4 favorecen el establecimiento y mantenimiento de la preñez. Contradictoriamente, hay investigaciones que no han hallado relación proporcional entre el tamaño del CL y la concentración de P_4; sin embargo si es claro el papel importante que desempeña la P_4 en el mantenimiento de la preñez a través de mecanismos antiluteoliticos y la estimulación de producción de IFN-t en el conceptus (Binelli et al., 2001; Mann, Lamming, Robinson, & Wathes, 1999).

Carnelli y colaboradores, en un estudio realizado en receptoras de embriones *in-vitro*, tuvieron una TP general de 58,1% (137/236), reportaron que receptoras cíclicas o que presentaban celo tuvieron una TP mayor 62,4% (106/170) que aquellas que no mostraban comportamiento de celo 47% (31/66) (P<0,01), el área del CL no fue diferente en receptoras gestantes y no gestantes, aunque la concentración de P4 fue mayor en las receptoras gestantes (P=0,04). Los autores mencionan que hay una correlación positiva de la presentación de estro, como manifestación de la ciclicidad ovárica con la TP en las receptoras de embriones, ya que exposición a estradiol durante el estro y P4 durante el diestro, están implicados en la preparación del útero para el gestación, el óptimo desarrollo embrionario temprano y el posterior reconocimiento materno de la gestación (Carnelli et al., 2014). En el 2009, Oyuela, en un estudio realizado en ganado cebú Brahman no observó diferencias entre animales con CL<10mm (28% TP), comparados con aquellos con CL entre 10-14mm y CL>14mm donde la TP reportada fue del 39% y 30%

respectivamente (P>0,05) (Oyuela, 2009). De manera contraria, Nogueira y colaboradores observaron en receptoras de embriones, que la TP se vio influenciada por el diámetro del CL (P<0,05) (Nogueira et al., 2012). Un estudio retrospectivo de 1399 TE de una central genética en Santander, reporto que receptoras de embriones PIV con un CL mayor a 20mm de diámetro tenían una TP mayor (32.06%) que aquellas que tenían un CL menor a 20mm de diámetro (30.53%) (Holguín, Montaña, & Valbuena, 2013). De la misma manera en Peru, Perez y colaboradores encontraron relación significativa del diámetro del CL con la TP en receptoras de embriones PIV, siendo que receptoras preñadas tuvieron CL de mayor diámetro al momento de la TE (19,54 +/- 1,33 mm), mientras que las receptoras no gestantes tenían CL de menor diámetro (16,86 +/- 2,47 mm), lo cual indicaría que hay una inferencia positiva del tamaño del CL, con la funcionalidad de este, los autores señalan que se puede debe a una mayor vascularización y una posible relación con la produccion de P4 (Perez Durand et al., 2022). Sin embargo la detección de un CL con una gran área de tejido luteal no garantiza que la concentración plasmática de progesterona sea alta, puesto que hay otros factores fisiológicos involucrados en el metabolismo hormonal que influyen en la concentración sistémica de progesterona (Siqueira et al., 2009).

Factores Asociados con el manejo de la trasferencia Embrión

En diferentes investigación sobre TE/PIV se ha evaluado la inferencia de multiples factores sobre el éxito de la TE, tales como el tiempo de duración de la TE, la persona que realiza la transferencia y el grado de dificultad durante el procedimiento de la TE los cuales pueden influir sobre la supervivencia del embrión y por consiguiente sobre la TP (Scenna et al., 2005). Un estudio Colombiano, realizado en el 2009 no encontró efecto (P>0,05) del técnico que realiza la transferencia, pero si para la dificultad en la ET, teniendo en cuenta que este procedimiento puede generar traumatismos en el endometrio (P<0,001) (P/ET con dificultad 11% vs. P/ET sin dificultad 39%) (Oyuela L.A, 2009). Otro estudio realizado en la Universidad de Tenesse, USA, evaluó la influencia de algunos factores sobre la TP

en 256 receptoras de embriones en donde se encontró que la persona que realizaba la TE no tuvo influencia sobre la tasa de preñez (P=0,75), mientras que la dificultad (P=0,02) en la TE, la localización del embrión transferido (P=0,04) y el tiempo (P=0,009) que duro la TE si tuvieron efecto sobre la TP. El autor reporta que hay una relación entre la duración de la TE y la manipulación del tracto reproductivo, ya que las TE que demoraron menos tiempo (6-9min) tuvieron mejores TP (60%), adicionalmente los embriones depositados en el tercio distal del cuerno uterino ipsilateral al CL tuvieron una TP del 60% en comparación con los embriones depositados en el tercio medio y craneal (37% y 35%). Concluyendo que los embriones que se depositan en la parte más distal del cuerno uterino tienen mayor probabilidad de preñez, sin embargo se debe evitar una manipulación excesiva del tracto reproductivo, lo cual puede generar producción de PGF2α y por lo tanto evitar que la preñez ocurra (Roper et al., 2018). Estos resultados son congruentes con un estudio realizado en Polonia, en donde evaluaron la relación del tiempo de la TE con la TP en 248 receptoras de embriones, los autores hicieron la medición del tiempo de paso del cervix y del tiempo hasta la deposición del embrión, siendo que cuando el tiempo fue de 20 a 60 segundos la TP fue 53,4%, si el tiempo era superior a 1 minuto la TP fue de 20,4% (P<0,01) (Jaśkowski, Urbaniak, Antosik, & Włodarczyk, 2010).

El autor Erdem y colaboradores, en un estudio realizado en un programa de transferencia de embriones reportaron una TP de 39,15% (242/618) y 35,11% (217/618) a los 30 y 60 días post transferencia y un porcentaje de perdida gestacional entre los 30 y 60 días de gestación de 10,33% (25/242). Los autores reportaron influencia de la calidad embrionaria sobre la TP, siendo que los embriones de “calidad 1” (calidad excelente y/o buena) tuvieron una TP de 44,15% (155/351) y 40,74% (143/351) a los 30 y 60 días post transferencia, mientras que embriones de “calidad 2” (calidad regular) tuvieron una TP de 32,58% (87/267) y 27,71% (74/267) a los 30 y 60 días post transferencia (P<0,05). La pérdida gestacional en relación con la calidad embrionaria fue de 7,74% (12/155) y 14,94% (13/87) para los embriones de calidad 1 y 2 respectivamente (P<0,05). Los autores relacionan los resultados obtenidos de la calidad embrionaria sobre la TP y la supervivencia embrionaria con el adecuado desarrollo embrionario y capacidad de supervivencia de los embriones, la cual está relacionada con la capacidad embrionaria de producción de Interferón trofoblástico bovino (bINT-t) durante en reconocimiento materno de la preñez en la

gestación temprana (Erdem et al., 2020); estos resultados concuerdan con lo reportado por Marzouk y colaboradores en donde las receptoras que recibieron embriones de primer grado tuvieron mayor TP (79,17%) que las que recibieron embriones de segundo y tercer grado, cuya TP fue de (48,94%) (P<0,05) (Marzouk, Genedy, Abdel-Razek, Zaghloul, & Elbaz, 2020) de la misma manera en Peru, hay un reporte de transferencia de embriones in vitro en 50 receptoras Brown Swiss, en donde la TP fue del 26% (13/50) y el 39% de las preñeces fue de embriones grado 1 (11/28) versus (2/22) de grado 2 (P<0,05) (Perez Durand et al., 2022).

El tipo de semen usado en la producción de embriones *in-vitro* también ha sido evaluado en relación con la TP, Mikkola y colaboradores, en un estudio retrospectivo de 12438 transferencias de embriones evaluaron el efecto del uso de semen sexado en la producción de embriones PIV, se realizaron 10.697 TE de embriones producidos con semen convencional, cuya TP fue de 44,1%, y 1741 TE de embriones producidos con semen sexado, cuya TP fue de 38,8%. Las transferencias de embriones fertilizados con semen sexado disminuyó la TP en aproximadamente un 12%, en relación con los embriones producidos con semen convencional (P<0,005) (Mikkola, Andersson, & Taponen, 2015).

Diversas investigaciones han dejado en evidencia el efecto que tienen múltiples factores sobre la TP y la supervivencia embrionaria en receptoras de embriones, sin embargo en la industria nacional se tiene pocos datos que correlacionen el impacto de estos sobre el éxito de los programas de transferencia de embriones, lo cual deja expuesta la necesidad de investigación del impacto que tienen factores ambientales, extrínsecos e intrínsecos sobre los resultados de los programas de TE y sobre la supervivencia embrionaria.

Conclusión

Se ha determinado que hay factores que influyen sobre el éxito (TP) de programas de producción y transferencia de embriones, por tal motivo es importante conocer y tener evidencia de como estos factores afectan los programas de transferencia de embriones producidos a partir de fertilización in-vitro, lo cual puede repercutir positivamente en tener cada vez mejores resultados en estos programas de transferencia de embriones.

En la actualidad se tiene amplio conocimiento de la presencia de enfermedades infecciosas reproductivas en los hatos bovinos y su fuerte implicación en problemas reproductivos, especialmente en la hembra bovina, lo cual repercute fuertemente en la eficiencia reproductiva y productiva de las explotaciones ganaderas. A pesar del amplio conocimiento y reportes que existen de la influencia de enfermedades infecciosas reproductivas sobre los hatos bovinos, no hay evidencia clara de la magnitud del efecto de estas sobre las receptoras de embriones bovinos producidos *in-vitro*, el efecto sobre la tasa de preñez y por ende en perdidas embrionarias y presentación de abortos.

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2. Risk factors for pregnancy outcomes and embryo survival in Bovine recipients of an *in vitro* produced (IVPE) embryo program in Colombian herds

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Abstract

The world production of bovine *in vitro-derived* embryos (IVPE) has had a vertiginous increase over the years as a tool for genetic improvement. Reported pregnancy rates range from 30.3-41.2 %. The variability of the pregnancy outcome of these programs may be influenced by intrinsic and extrinsic factors, that are related to the recipient. Consequently, the objectives of the present study were to evaluate the effect of the health status of embryo recipients, as well as the influence of intrinsic and extrinsic factors that may affect pregnancy outcomes and embryo survival in a regional *in vitro* embryo transfer program. Small and medium-sized producers from 34 municipalities in the department of Cundinamarca (Colombia) were invited to participate. Cattle operations included different

climates and thermic floors as well as dairy beef and mixed production systems. All animals were subject to clinical and reproductive examinations followed by blood sample collection for the assessment of infectious pathogen exposure, hepatic and renal function, and hematologic & coprological examinations. Test-positive animals for B. abortus, N caninum, and acute VBVD infections were excluded from the program. All recipients were subject to estrus synchronization protocols for FTET. Logistic regression models were performed to assess causal associations between the study variables and the pregnancy outcome and the embryo survival rate. The magnitude of the association was estimated through the Odds Ratio with a 95% CI and $p<0.05$. A total of n=5708 animals were examined and sampled as potential embryo recipients in this program. Out of these, a total of n=4121 (72.2%) were enrolled in the study according to the criteria for inclusion. The average pregnancy rate in this study was 26.1%. Embryo recipients who did not present changes in the results of clinical pathology associated with fibrimogénemia, neutrophilia, and eosinophilia had a higher probability of having a viable gestation (OR=1.5 CI 95% 1.1-2.1, p=0.025); OR= 1.9, 95% CI 1.0-3.4, p =0.035) and OR= 2.2 IC 95% 1. 5 – 3. 5, p=0.001, respectively). Intrinsic and extrinsic variables affecting pregnancy outcomes were: vaccination against reproductive diseases (OR= 0.4, 95% CI (0.2-0.6), p= 0.001), the use of conventional semen compared to sexed semen (OR = 0.6, 95% CI 0.4-0.9, p = 0.009, and Cyclic animals at the time of estrus synchronization for ET (OR=1.2, 95% CI 1. 1 – 1.5, p=0.025). The overall prevalence of gestational loss was 8.3%. An increased risk of gestational loss due to exposure to BoHV-1 (OR= 5.4, 95% CI 1.1-27.6, p =0.043), and *to L hardjo bovis* and *L canicola* (OR= 4.0, 95% CI 1.1-14.9, p=0.032), and (OR= 8.9, 95% CI 1.6-14.0, p=0.021), respectively. Animals that had a significant decrease in BCS from the time of ET to the pregnancy diagnosis showed a higher risk of suffering gestational loss (OR= 3.6, 95% CI 1.1-12.8, p =0.048), compared to those who maintained BCS Also, increased BCS was protective for embryonic survival (OR= 0.16, 95% CI 0.05-0.53, p=0.002). It was estimated that those animals with normal body temperature < 38.5 °C at the time of TE, were at a lower risk of gestational loss compared to those with body temperatures > 38.5 °C (OR = 0.1, 95% CI 0.007-0.8, p = 0.03). Recipients exposed to ITH thermal comfort zones > 68 had a lower risk of suffering gestational losses (OR= 0.023, 95% CI 0.001-0.775, p =0.036). Our findings highlight the importance of health and infectious disease assessment as well as reinforce biosecurity and animal welfare conditions as a pivotal role in the election of embryo recipients to enhance pregnancy outcomes and embryo survival in an *in vitro* embryo transfer program.

Keywords: Gestation, Odds Ratio risk, risk factors, livestock, embryo survival (Sources: DeCS, AGROVOC Multilingual Thesaurus, Thesaurus animal biology).

Introduction

The world production of bovine *in vitro-derived* embryos (IVPE) has had a vertiginous increase over the years as a tool for genetic improvement. According to The International Embryo Technology Society (IETS) in 2020, out of a total of 223,620 oocyte donors, 4.120,754 oocytes resulted in 1.132,773 transferable embryos within the five continents, 42,6 % of which (483,587) were produced in South America. In Colombia, the use of IVPE embryos has also increased significantly in recent years. However, success reports measured with the blastocyst production rate are scarce [2]. Reported pregnancy rates (PT) range 30.3-41.2 % [3-5]. The variability of the outcome of these programs may be influenced by different factors. Different studies have shown that the success of IVPE programs could be affected by intrinsic factors, that are directly related to the recipient, such as health status, exposure to infectious agents, and quality of the corpus luteum (CL), among others. Also, there are extrinsic factors, such as environmental conditions, or those directly related to the embryo and the embryo transfer (ET) technique. Other factors that can influence PT in female bovine recipients have been studied such as boy condition (BCS) as an indicator of nutritional status that can impact reproductive health [6, 7]. Also, corpus luteum volume and its relationship with serum concentration of progesterone (P4). It has been reported that the concentration of P4 is proportional to the size of the CL which is important given that P4 favors the establishment and maintenance of gestation through anti-luteolytic mechanisms stimulated by IFN-t production by the conceptus [7, 8]. Likewise, factors associated with the ET technique have been described, such as the site of deposition of the embryo within the uterus, timing, duration, and difficulty of the transfer [8, 9] [10], as well as the influence of the quality of the embryo[11]. Furthermore, it has been reported that the quality and the state of development, as well as the breed of the embryos at the time of transfer, have been associated with the ability to undergo a morphological transformation, elongation, and production of INFt for the correct signaling for pregnancy recognition and implantation[12]. Additionally, multiple infectious causes have been previously studied that may affect embryo survival[13]. The present study aimed to evaluate the effect of the health status of recipients, as well as the influence of intrinsic and

extrinsic factors that may affect pregnancy outcomes and embryo survival in a regional *in vitro* embryo transfer program.

Materials and methods

Study Population

A target population of small and medium-sized producers from 34 municipalities in the department of Cundinamarca (Colombia) was invited to voluntarily participate in the study. Cattle operations included different climates and thermic floors as well as dairy beef and mixed production systems. The sample size was estimated based on the number of heifers between 2- 4 years of age with the potential to be eligible as embryo recipients (n=31149) based on this population and calculating that at least 35%, were readable for ET, with a confidence limit of 5%, a design effect of an estimated pregnancy percentage of 25%, and a study power of 80%; a sample size of n=3725 animals was estimated, with an additional 10% increase was considered accounting possible involuntary loss of animals for a total of n=4084 embryo recipients (Epinfo).

Herd Selection

Through the regional livestock associations, farms were selected with a minimum of 5-50 bovine females (2-3 years or 3-4 years if there is no availability of young animals) suitable for embryo transfer, and that had a minimum of nutritional, management and welfare requirements for the animals; in the same way, that the execution of the activities throughout the project was guaranteed. An initial characterization visit of the property was carried out by Veterinarians who collected data related to the inventory of animals, health, and reproductive management information, and who also performed both clinical and reproductive examinations of the animals. Criteria for inclusion included: heifers without anatomical and reproductive abnormalities, reaching a minimum weight of 65-70% of adult herd weight. Additionally, good health, without reproductive pathologies, and a body

condition score (BCS) between 3.0 and 4.0 (on a 1/5 scale). Subsequently, a sample of whole blood and serum by coccygeal venipuncture was collected from all potential recipients and submitted to the Laboratorio Clínico FMVZ Universidad Nacional de Colombia for further analysis. First, whole and serum blood samples were processed for hematological analysis as well as for hepatic, renal, and protein metabolism assessment. In addition, all the blood serum samples were subject to an indirect enzyme-linked immuno-absorption assay (ELISA) to evaluate the level of exposure to *Bovine Herpes Virus type 1* (BoHV-1), *Neospora caninum, Bovine Enzootic* Leukosis *Virus (BELV), and Brucella abortus.* An antigen capture *ELISA* test was also used to detect acute infections from Bovine *Diarrhea Virus* (BDV). Also, a microscopic agglutination test (MAT) for serological diagnosis of Leptospira serovars (*L. hardjo prajitno*, *L. hardjo bovis, L. pomona, L. canicola, L. grippotyphosa L. icterohaemorrhagiae)*. All those animals that tested positive for *Neospora caninum,* BVD-Ag, and *Brucella abortus* were excluded from the study, considering the risk of vertical transmission during pregnancy, particularly, in the case of *B abortus* because it is a reportable disease under an official control program in the country.

Recipient estrous synchronization and Embryo Transfer

The selected recipients were subjected to two types of heat synchronization protocols to ET depending on their geographical and altitude location. The recipients located under warm-hot weather (altitude lower than 50-1600 Meters Above Sea Level (MASL) received 2.5mg of estradiol benzoate and received an intravaginal device of progesterone DIV (Sincrogest®) at day 0, at day 7 0.53 mg of Prostaglandin F2alpha (Sincrocio®) and 400 IU of Equine Chorionic Gonadotropin (Sincroecg®) IM were injected, on day 8 the progesterone implant was removed, at day 9, 1.5mg of estradiol benzoate IM was injected, on day 10 heat detection was performed and on day 18 transfer of the embryos was performed. In contrast, those recipients located in cold weather regions (altitude higher than 1600 MASL) were injected IM with 0.0105mg of Buserelin Acetate (Sincroforte®) and simultaneously received an intravaginal device of progesterone DIV (Sincrogest®) at day 0, on day 7, an IM injection of 0.53mg of Prostaglandin F2 alpha (Sincrocio®) was performed, and the progesterone implant was removed on day 8. On day 9, another IM dose of 0.0105mg of Buserelin Acetate (Sincroforte®) was injected, on day 10 heat behavior was detected, and on day 18 embryo transfer was performed. On the day of the

ET, the presence of a CL was evaluated by rectal palpation and verified by ultrasound as a criterion for ET. The recipients were restricted and received an epidural injection of 5 ml of 2% lidocaine, perineal asepsis was performed and transcervical transfer of the embryo was carried out, placing the embryo on the ipsilateral uterine horn to the corpus luteum. Data collection at the time of the transfer of the embryos included: the racial type of the recipient, BCS, corpus luteum, volume (CLV), site of the transfer within the uterus, depth of the delivery, duration of the transfer (min), number of ET transfers, operator who performed the transfer, and the degree of difficulty. Also, the breed of the embryo, quality, and stage of embryonic development, body temperature (Cº), and the current environmental temperature (Cº). Pregnancy diagnosis was performed using transrectal ultrasonography (SonoScape E1, SonoScape Medical Corporation) with a linear transducer of 5-7 MHz two times. First, between 30-45 days following TE, and then, a reconfirmation exam was performed between 60-90 days. A diagnosis of viable gestation was considered when the viable presence of an embryo or fetus and fetal heartbeat was observed. The reconfirmation of pregnancy viability was carried out to evaluate the occurrence of gestational losses.

Gestational Loss: Definition and Classification of Cases

Case definition and case classification were previously reported [14]. The gestational loss was considered when any of the following events occurred after gestation diagnosis: 1. Expulsion of the fetus and fetal membranes, 2. Presentation of estrus 3. If no signs of viable gestation at the time of examination between days 60-90 post-transfer were observed at the time of the reproductive examination.

Data collection.

Information from the geographical location of the animals enrolled was collected as well as the results of both clinical and reproductive examinations. In addition, genetic and environmental data were categorized to facilitate the analysis.

1. Geographical distribution: Municipality and province.

2. Health Aspects: Albumin (gr/L), BUN (mg/dL), Creatinine (mg/dL), GGT (U/L), AST (U/L), Leukocytes (10^3/µl), Lymphocytes (%), Monocytes (%), Neutrophils (%), Eosinophils (%) PCV (%), PPT (g/dL), Fibrinogen (mg/dL), Additionally, the results of serological tests (LVB*, BOHV-1, L grippothyphosa, L canicola, L Pomona, L hardjo prajitno,L. icterohaemorrhagiae L hardjo bovis)* and *BVD* acute infection, and last, the degree of parasite infestation (EPG).

3. **Intrinsic and intrinsic aspects of the recipients:** Recipient age (months), Estrus synchronization protocol, BCS at estrus synchronization, recipient ovarian cyclicity status, body temperature (Cº) at estrus synchronization, body temperature (ºC) at the time of ET, body temperature (Cº) at pregnancy diagnosis. Degree of difficulty of ET, Embryo seeding site, Time of the duration of the ET (min), BCS at the time of ET, Change in BCS at pregnancy diagnosis, vaccination status against reproductive pathogens, Type of semen (conventional vs sexed), Corpus Luteum Volume (mm3) at the time of transfer (TE).

4. Genetic features: Recipient racial type, Embryo breed, bull breed or bull species, donor cow breed.

5. Environmental aspects: Environmental temperature (month of ET), Relative humidity (%), rainfall (mm ^{3}), Humidity Temperature Index (%), a calculated as ITH= (1.8 * TA + 32) - ((0.55–0.0055 * HR) x (1.8 * TA - 26)), where TA denotes the ambient temperature and H R is the relative humidity[15].

A data collection template based on the initial results was designed and standardized to facilitate and unify the collection of information by the Veterinarians who participated in the study.

Statistical analysis

Descriptive statistics were performed on all continuous variables (results of hematology, blood chemistry, and serology of infectious diseases, as well as the age and weight of the

recipients to establish their frequency of presentation, and to assess the normal distribution of the data. The information collected on seropositivity status to infectious reproductive diseases, hematological variables, blood chemistry, parasitology, general clinical examination, reproductive examination, hormonal synchronization, embryo transfer, pregnancy diagnosis, and reconfirmation of gestation was tabulated and categorized. The prevalence of exposure to each of the reproductive infectious diseases was estimated considering the number of positive cases out of the total samples analyzed and the result was expressed as a percentage. The data were analyzed first using a univariate analysis where the independent variables presented against the pregnancy outcome were cross-analyzed. Each factor was analyzed by using 2x2 contingency tables using the chi-square test. Following the Hosmer and Lemeshow criteria [16] all variables with a pp-value< 0.25 in the univariate model were analyzed through Binary Logistic Regression (OR= exp (Xb) / [1 + exp (Xb)]). The strength of the association between pregnancy outcomes and the different risk factors was estimated through the Odds Ratio (OR) with a 95% confidence interval and a P value < 0.05. The data were analyzed with the statistical package SPSS-IBM version 21.0.

Results

Characteristics of the embryo recipients

A total of n=5708 animals located in 34 municipalities of the Department of Cundinamarca, (Colombia) and located on different thermal floors were examined and sampled as potential embryo recipients in this program. Of these, a total of n=4121 (72.2%) were enrolled in the study according to the criteria for inclusion. Out of the total, 18.1 % were younger than 24 months, 47.1 % were between 24 -48 months, and 26 % were older than 48 months. Regarding the racial type, 22.1 % n = 911 were *Bos taurus,* 27.7 % (n = 1143) were *Bos indicus,* and the remaining 50.2 % (n = 2067) were crossed breed animals. It is very important to note that, although all animals had been vaccinated as part of the official control program against Foot and Mouth Disease and Bovine Brucellosis, only 12.0 % reported the use of vaccines against reproductive pathogens. Out of the excluded animals, 1.1% showed acute *BVD* infections, 7. 8 % and 3.5% were seropositive to *Neospora*

caninum, and *Brucella abortus* respectively, accounting for a total of 696 (12.2%) animals discarded due to the risk of placental transmissible infection during pregnancy. Regarding exposure to other pathogens, 45.1% of the animals tested positive for *BoHV-1* and 23.0 % for *BEL*, while positive results were obtained for different leptospira serovars range 10.0 - 23.1% (*L. hardjo prajitno* 23.1 %, *L. canicola* 18.6 %, *L. hardjo bovis* 18.0%, *L. pomona* 17.6 %, *L. grippotyphosa* 12.0 %, and *L. Ictherohemorragie* 10.0%. Additionally, 1172 animals (20.5%) experienced health problems at the time of selection represented as elevated

Fibrinogen > 700 mg/dl (2.3 %), Leukocytosis >18000 (5.3%), Neutrophilia (0.07%), Lymphocytosis > 95% (0.18 %), eosinophilia (3.5%), high serum creatinine concentration (0.5%), BUN > 25mg/dl (2.6%), GGT > 48 IU (1.9%), Hypoalbuminemia > 2.2 (0.36%), Hypoproteinemia < 6.0 gd/ml (0.9%), PCV< 20% (0.6%), PCV > 45 (1.9%) . In addition, 53.0% of the animals showed of gastrointestinal parasites infestations that included Nematodes (33.2%), Eimeria (12.3%), Trematodes (2.5%), protozoa (1.7%), and Cestodes (0.6%). Out of these, 28.8 % showed mild infestation (<200 e.p.g), 17.9% showed moderate infestation (200 - 1000 e.p.g,), and 3.6% showed severe infestations (> 1000 e.p.g.) In all cases, the Veterinarians made the relevant recommendations based on the results of clinical and laboratory examinations, to initiate the heat synchronization treatments for fixed-time- embryo transfer (FTET).

Characteristics of the TE

All embryos produced were transported to the farms reaching grade 1 blastocyst status to be transferred between days 6.5 and 7.0. Two heat synchronization protocols were used depending on the racial type of the recipients and their location in cold weather (21.1% or warm 79. 9%) the ovarian cyclic status of the recipient at the time of heat synchronization was also considered 49.4 vs 49. 6% cyclic vs anovulatory status respectively. Embryos were transferred if a corpus luteum > 10 mm in diameter was evidenced after the clinical reproductive examination. The average CL Volume was 4097.0±827.29 (mm^3), (range 827.3 to 9780.9 mm^3). Moreover, the transfer time (min) after epidural anesthesia was less than 5 minutes in 89.6% of the cases and longer than 5 min in 10.3% of them. The depth of the embryo seeding was at the base of the uterine horn in 25.5% of the cases, in the middle third of the horn in 73.3%, and the cranial third in 1.1% of cases. The operator who

performed the TE was also duly identified. Information was collected from environmental variables since the recipients were selected. The average monthly rainfall (mm) from the time of ET until the second pregnancy diagnosis was 128.5 ± 61.8 mm, (Range 28-380 mm). Likewise, the monthly average Relative Humidity (%) was 75.5% ± 10.4 (Range 31.9-99.0%), and the Temperature Humidity Index (ITH) was on average 67.7 ±7.9 (Range 51.0 - 85.9). Furthermore, environmental temperature (C°) at one day of ET was on average 20.9 °C ± 5.7 (Range 10-33°C). Regarding genetic features, embryos of twelve different breeds were transferred (Zebu Brahman, Zebu Nelore, Zebu Guzerat, Zebu Gyr, Bradford Brangus, Gyrolando, Holstein, Jersey, Normandie, Simmental, and Simbrah. Following ET, a total of 1044 recipients showed a positive viable diagnosis of gestation, which corresponded to an average PT of 26.1 % (Range 0.0-42.0 %), Data from one hundred and twenty-eight animals that were already ET were removed from the study analysis because it was not possible to perform pregnancy diagnosis since they were moved to other farms, sold, or the owners did not allow the pertinent diagnosis. A total of 56.0% of the recipients conceived at the first ET, 2and 8.9% and 15.1% conceived at the second and third transfers respectively.

Factors associated with pregnancy outcomes

This is the first report to describe critical aspects of bovine embryo recipients' selection based on their health status. Particularly, avoiding the use of recipients previously exposed to some known vertically transmitted pathogens that may cause embryonic and fetal loss, and therefore reducing embryo survival, as a contribution to disease control.

The average pregnancy rate in this study was 26.1%. It was observed that embryo recipients who did not present changes in the results of clinical pathology associated with fibrimogénemia, neutrophilia, and eosinophilia had a higher probability of having a viable gestation compared to those animals with abnormal results (OR=1.5 CI 95% 1.1-2.1, p=0.025); OR= 1.9, 95% CI 1.0-3.4, p =0.035) and OR= 2.2 IC 95% 1. 5 – 3. 5, p=0.001, respectively) (Table 1).

Similarly, intrinsic and extrinsic variables included in the final regression model were: vaccination against reproductive diseases (OR= 0.4, 95% CI (0.2-0.6), p= 0.001) and the use of conventional semen compared to sexed semen (OR = 0.6, 95% CI 0.4-0.9, p = 0.009), both factors favored the conception and establishment of a viable gestation in this study, Furthermore, cyclic animals at the time of estrus synchronization for ET (OR=1.2, 95% CI 1. 1 – 1.5, p=0.025) had a higher likelihood of conceiving compared to those who presented anovulatory conditions at the beginning of the estrus synchronization program. Also, those recipients whose CL Volume at the time of ET was between 3000 - 5000 mm^3 (OR = 1.9 95% CI 1.0-3.8, p=0.05) had a higher chance of carrying a normal pregnancy. On the other hand, analysis of environmental factors showed that the ambient temperature (°C) as an indicator of animal comfort showed that the exposed recipients to temperatures that could induce caloric stress > 25 ºC had a higher risk of negatively affecting the outcome of a viable pregnancy (OR= 1.6, 95% CI 1.2-2.2 P=0.005) compared to those exposed to a thermoneutral zone (15-25 ªC), and those subjected to a lower ambient temperature (< 15ºC). Also, those animals that living in areas of Humidity Relative (%) with a comfort rate between 60-75 %, had a higher pregnancy probability (OR = 1.9, 95% CI 1.3-2.7, p = 0.001) compared to those subjected to a relative humidity < 60% and > 75%. (Table 1). Moreover, no association was observed with exposure to pathogens that affect bovine reproduction or with factors associated with genetics such as the breed of the embryo, the racial type of the recipient, or the breed of the bull (*Bos taurus* vs *Bos indicus* or their crosses (P > 0.05), but embryos from donors *Bos indicus* (OR=1.9, 95% CI 1.1-3.2, p=0.045 had almost two times more probabilities of establishing a viable gestation compared with embryos from other breeds. Additionally, interaction terms used in the regression models were included in the final model.

Table 1: Factors associated with the diagnosis of a viable gestation in bovine recipients of *in vitro-produced* embryos (IVPE) in a regional program in Colombian herds

	Variable	Category	n	OR	IC 95% OR		P-value
					Lower	Upper	
HEALTH	Neutrophils (%)	0-42 %	300	1.5	1.1	2.1	0.025 *
		Greater than 42 %	872				
	Eosinophils (%)	0 - 10 %	1114	1.9	1.1	3.4	0.035 *
		11-55%	58				
	Fibrinogen (mg/dL)	Less than or equal to 700 mg/dL	1048	2.2	1.5	3.5	0.001 *
		Greater than 700 mg/dL	124				
INTRINSIC AND EXTRINSIC	Reproductive diseases control	No Vaccination	980				
		Vaccination	192	0.4	0.2	0.6	0.001 *
	Ovarian Cyclicity	Anovulation	1296				
		Cyclic	1384	1.2	1.1	1.5	0.025 *
	Type of Semen	Sexed	725				
		Conventional	447	0.6	0.4	0.9	0.009 *
		< 3000 mm3	80				
		3000 - 5000 mm3	943	1.9	1.0	3.8	0.05 *

	Corpus Luteum Volume (mm3) at TE	> 5000 mm3	149	1.2	0.7	2.0	0.578
	Operator TE	1	178				
		2	820	1.2	0.8	1.8	0.034 *
		3	174	0.59	0.36	0.95	0.029 *
GENETIC	Donor Species	*Bos taurus*	197				
		Bos indicus	938	1.9	1.1	3.2	0.045 *
		Bos taurus x Bos indicus	37	0.5	0.1	1.6	0.291 *
ENVIRONMENTAL	Ambient temperature at ET (Animal comfort)	Warm Zones> 25ºC	389				
		Cold Zones< 15ºC	315	1.1	0.8	1.3	0.652
		Thermoneutral Zone(15-25 ªC)	468	1.6	1.2	2.2	0.005 *
	Relative humidity (%)	< 60 % HR	113				
		Animal Comfort range 60-75 %	342	1.9	1.3	2.7	0.001 *
		> 75 % HR	717	1.1	0.9	1.4	0.313

n= animals OR: Odds Ratio, 95 % Confidence Interval, * p-value < 0.05 indicates statistically significant differences. A total of 37 variables analyzed were excluded by the final model.

Risk factors associated with gestational loss

The overall prevalence of gestational loss observed in this study was 8.3%. An increased risk of gestational loss due to exposure to pathogens such as BoHV-1 (OR= 5.4, 95% CI 1.1-27.6, p =0.043), and *to L hardjo bovis* and *L canicola* (OR= 4.0, 95% CI 1.1-14.9, p=0.032), and (OR= 8.9, 95% CI 1.6-14.0, p=0.021), respectively was estimated. Other health variables evaluated were not included in the final regression model. Likewise, from the intrinsic and extrinsic factors that could be associated with the occurrence of gestational loss, it was observed that those animals that had a significant decrease in BCS from the time of ET to the second pregnancy diagnosis showed a higher risk of suffering gestational loss (OR= 3.6, 95% CI 1.1-12.8, p =0.048), compared to those who maintained BCS, and also, that the increase in BCS could be a protective factor for embryonic survival (OR= 0.16, 95% CI 0.05-0.53, p=0.002). Very similar, it was estimated that those animals with normal body temperature < 38.5 °C at the time of TE, were at a lower risk of gestational loss compared to those recipients with body temperatures > 38.5 °C (OR = 0.1, 95% CI 0.007-0.8, p = 0.03) (Table 2). Similarly, a causal association with some environmental variables may be established. Recipients exposed to ITH thermal comfort zones > 68 had a lower risk of suffering gestational losses (OR= 0.023, 95% CI 0.001-0.775, p =0.036) (Table 2). Finally, none of the variables of the genetic features studied showed a statistically significant association in the logistic regression model.

Table 2. Risk Factors affecting embryonic survival in bovine recipients in a regional *in vitro* produced embryo (IVPE) program in Colombian herds

	Variable	Category	n	OR	IC 95% OR		P-value
					Inf	U	
Health	BoHV-1	Negative	162				
		Positive	99	5.4	1.1	27.6	0.043*
	L hardjo bovis	Negative < 1:100	214				
		Positive >1:100	47	4.0	1.1	14.9	0.042*
	L. canicola	Negative < 1:100	215				
		Positive >1:100	46	8.9	1.6	14.0	0.021*
Intrinsic-Extrinsic	BCS Change (ET-Dx Gestation)	There was no change	139				
		Loss of BCS	61	3.6	1.0	12.8	0.048*
		Improving BCS	61	0.16	0.05	0.53	0.002*
	Body Temperature (Cº)at TE	> 38.5ºC	32				
		< 38,5 ºC	229	0.1	0.007	0.8	0.030*
Environmental	Temperature-Humidity-Index	ITH > 68 – 72 Thermal Non-Comfort Zone	163				
		ITH < 68Thermal Comfort Zone	98	0.023	0.001	0.775	0.036*

n= animals OR: Odds Ratio, CI= 95% Confidence Interval of OR, * p-value < 0.05 indicates statistically significant differences.

The variables: lymphocytes (%), level of parasite infection (e.p.g), Total Proteins (mg/m), Age of the recipient (months), (Corpus luteum volume mm ^{3}), Breed of the embryo, Donor and Bull Species, Environmental Temperature at transfer (ºC), Ambient temperature at ET (Animal comfort), Rainfall (mm), monthly temperature after ET, and Relative Humidity (%) were not statistically significant in the final logistic regression model p >0.05)

Discussion

The results of this research allow us to understand some of the factors that may be determinants of the occurrence of viable pregnancies in an *in vitro* embryo-produced program under different geographic, climate and health, genetics, and environmental conditions that may have influenced the recipients either directly or indirectly. First of all, it should be noted that the occurrence of a viable gestation was not influenced by the level of exposure to pathogens that typically affect reproduction as has been the case with the vertical transmission of BVD [17], or that of *N caninum,* where it has been proposed that the use of seronegative embryo recipients is an effective method of controlling congenital infections [18], and therefore, this is a suitable strategy of controlling fetal infections and controlling disease transmission. Similarly, the prevalence of gestational losses has been reported to be higher in animals seropositive to *B. abortus*[19], although logically these animals must be removed from the population as part of the official disease control program, therefore, their identification and elimination must be part of the selection criteria of embryo recipients, among others. Additionally, a protective effect was observed from the use of vaccines against reproductive pathogens, despite their limited use observed in this study (12.0%). Vaccination has been reported to be effective to control the reproductive effects caused by VBDV and BoHV-1 [20] or with the addition of 5-7 leptospira serovars [21],[22] which have resulted in a better pregnancy rate in animals undergoing Fixed-Time-Artificial-Insemination programs (IATF) [22]. Together these results reinforce the need to

implement and target disease control programs more specifically, particularly in embryo transfer programs.

On the other hand, some systemic infections have previously been reported as a cause of gestational loss in bovine embryo-recipient females[23]. The occurrence of diseases has been associated with common factors such as negative energy balance (BEN) and alterations in the immune system function [24]. Overall, diseases and inflammatory conditions can affect the establishment of a healthy gestation in part due to the disruption in maternal-fetal endocrine signaling [25]. Moreover, cattle experience frequent immunological challenges, where the presentation of diseases that have typically been reported to affect the establishment of a new pregnancy or to cause embryo o fetal loss include metritis [26] and mastitis[27], among others, whose occurrence was not observed in this study since heifers were mostly used as recipients. However, it is important to consider other more discrete inflammatory conditions such as increased gastrointestinal permeability, which can be a consequence of different stressors, including dietary changes [28], systemic inflammation and endotoxemia [29], or environmental factors that may occur with hyperthermia which endangers animal welfare and may alter the metabolism of carbohydrates, lipids and proteins[30]. Particularly, the activation of the immune response begins when the molecular patterns of pathogens are recognized by immune cells, which causes the transcription and production of inflammatory cytokines resulting in an acute phase response characterized by fever, changes in the circulating number of leukocytes, and hepatic synthesis of acute phase proteins [31], and that may coincide with the increased serum concentrations of Fibrinogen and neutrophilia observed in this study, which could have limited the possibility of conception and the healthy development of a viable gestation [32]. Similarly, although this study did not find any association between the degree of infestation by gastrointestinal parasites and the pregnancy outcome, it was determined that those animals with higher-than-normal eosinophil percentages were at a higher risk of not accomplishing pregnancy following ET. It has been observed that, in cases of parasitism, especially those involving nematodes capable of invading tissues, a strong Th2-type immune response is induced, which increases the levels of immunoglobulin E and eosinophils in both blood and tissues [33]. [33] Therefore, parasitic infestations depending on the type of parasite, or the degree of an infestation can cause negative health effects that could additionally affect reproductive efficiency and pregnancy outcomes in cattle.

Although the method of estrus synchronization of the recipient was not a factor associated with the establishment of a viable gestation in this study, the ovarian cyclicity status of the recipient was a factor that favored pregnancy outcomes. Nevertheless, in this study, it was observed that the anovulatory conditions of the recipients had a negative influence on the establishment of a new gestation following ET. Anovulation is usual during postpartum, and its physiological basis has been previously described [34]. The impact of this condition has been such that it has been reported that the pregnancy rate of 21-d can be reduced by up to 65-83% of what is observed in cyclic animals [35]. Anovulatory conditions can influence the quality of the corpus luteum after estrus synchronization protocols for FTET, which was confirmed in this study where the normal volume of the CL (3000-5000 mm^3), also had a positive effect on the establishment of gestation. In this way, it has been observed that the hormonal and metabolic changes imposed by anovulation decrease the frequency of LH pulses [36], which generally affects folliculogenesis, and induces a high expression of inflammatory mediators in the endometrium[37] affecting the elongation and implantation of the conceptus [38]. These findings together highlight the importance of including cyclic animals as recipient selection criteria as a strategy to improve both responses to heat synchronization treatments and pregnancy rates in embryo transfer programs. On the other hand, analysis of the extrinsic variables associated with the management of the embryo at the time of transfer found no association with factors such as the level f difficulty of ET, Embryo seeding site, Time of the duration of the ET (min), this is possibly attributable to the expertise of the operators who performed the ET.

Genetic-associated causes that commonly affect the development of viable pregnancies and that may affect embryonic survival include chromosomal defects, failure to express some individual genes, lethal genes, and genetic interactions, as well as inbreeding [39]. None of these variables were the subject of study in the present, but it could be established that embryos from *Bos indicus* donors had a higher probability of gestation compared to the other species evaluated. It has been proposed that ET from breeds adapted to certain environmental conditions favors the pregnancy outcome [40]. Also, a favorable effect of the use of conventional semen on sexing was observed. It has been reported that sexed semen also compromises *in vitro* embryo production, as it impairs cleavage rate, blastocyst

production, and embryo viability after embryo transfer [41]. Otherwise, other studies have found no difference in pregnancy rates attributable to the breed of the donor [42], the racial type of the recipient, or the breed of the bull used in IVF, although in the latter case it is known that successful embryonic development depends on the genetic and epigenetic contribution of both male and female, possible adverse effects attributable to the male about embryo quality and development have probably often been undervalued [43].

In Colombia, the effect of environmental temperature on the establishment of gestation has not been elucidated. In this study, gestation was favored when the embryos were transferred to receptors located in zones of thermoneutral ambient temperature (15-25 ªC) compared to those subjected to temperatures greater than 25 ºC, but not compared to those in climates with temperatures less than 15 ªC. Important phenomena such as luteolysis, maternal recognition of gestation, and implantation, are regulated by prostaglandins (PG) secreted from the uterus[44]. There is a recognized effect of high ambient temperatures that can directly induce PG production in endometrial cells [45], which could contribute to the low rate of conception and pregnancy outcomes in exposed animals. Ambient temperature and relative humidity are critical determinants for the thermal comfort of the bovine. In this regard, a recent study defined that between days 24-31 of gestation there are more embryo losses which negatively impact the pregnancy rate during warm months compared to cold or winter months. This study also estimates that under these climate circumstances the secretion of progesterone (P4) is seriously compromised affecting the development of gestation [46], because the raise of the luteal progesterone during the early diestrus, ensures embryonic development and elongation of the conceptus [47]. Moreover, inadequate maternal levels of P4, or atypical progesterone profiles, may influence delayed placentation and placental insufficiency, which may be potential causes of embryonic death and, consequently, is an additional risk to the establishment of a viable gestation [48].

On the other hand, defining the risk factors associated with embryonic or fetal losses is useful for understanding this problem, adjusting the conditions that precipitate the loss, or applying approaches that allow for reducing the impact on high-risk animals. The rate of gestational loss observed in this study was 8.3% which compared to other studies reporting losses between 7.2-29% [49, 50], was relatively low. However, it was possible to establish

a causal association between gestational loss and exposure *to BoHV-1* and *leptospira serovars hardjo bovis and leptospira canicola*. The consequences of *BoHV-1* infection can range from abortion outbreaks affecting large populations of pregnant cattle to more subtle reproductive losses including low conception rate, embryonic mortality, premature births, and stillbirths[13]. Similarly, the effects of infection by different Leptospira serovars have been described, leptospirosis represents a silent and chronic reproductive disease characterized by embryonic death subsequently accompanied by heat repetition and subfertility at the herd level [51][51], among other reproductive and health effects. Many studies have focused on the assessment of the risk of disease transmission through assisted reproduction techniques and have concluded that the risk of infection is relatively low when following the health protocols recommended by the IETS, and adequate biosecurity control is achieved throughout the implementation of well-oriented vaccination programs, disease control of embryo donors [52]. However, the other counterpart of the risk of infection during pregnancy following embryo transfer is highly influenced by the health and management of the recipient groups. In this study we observed a low vaccination coverage against classic pathogens that affect reproduction in the group of animals who were diagnosed pregnant, only 12.0 %, this finding coupled with the observed wide exposure to pathogens determined through the study prevalence, highlights the need for implementation reproductive disease control and prevention programs that include better biosecurity: vaccination, quarantines, and sampling of recipients before TE or mobilization of before entry the herd o herds, given that both BoHV-1, Leptospira, and the pathogens that were used as criteria for recipient exclusion from this study (*N. caninum, BVD, and Brucella abortus*), among others, can cause acute, subclinical or congenital infections in both embryo recipients and their offspring, contributing to an increased risk of infection of the herds [53].

On the other hand, the loss of weight or BCS during the first trimester of gestation had a negative influence on embryonic survival. It has been reported that environmental or health situations that lead to a reduction in food consumption tend to increase negative energy balance and can alter not only ovarian and luteal function, but can also induce an imbalance activity of the hypothalamic-pituitary-ovarian axis, which can alter oviductal function leading to failure in fertilization and embryonic death [54]. Similarly, a negative effect on embryonic survival was evidenced when the recipient's body temperature was higher than 38.5 °C at

the time of ET, suggesting that hyperthermia, as previously reported, may affect the high sensitivity to high temperatures of both oocytes and embryos during the early stages of development [55]. Moreover, it has been shown that hyperthermia affects cell function at the level of the reproductive tract[56], which can induce increases in the endometrial secretion of PG E2 and PGF2α in cattle[57], increasing the risk of luteolysis, and therefore, decreasing embryonic survival. On the contrary, this negative effect was not observed in those animals that gained BCS during the first trimester of gestation and in those that were transferred in Thermal Comfort Regions (ITH < 68) which had a higher probability of embryonic survival. One study established that ITH is highly associated with cortisol concentration in embryo-recipient females and that animals with low cortisol concentrations have better pregnancy outcomes [58][58]. It has been possible to establish that animals under stress conditions stimulate with greater intensity the hypothalamic-pituitary-adrenal axis giving rise to neural-endocrine stress, which is characterized by the increase in the release of ACTH and cortisol[59], and that this hormone interferes with mechanisms related to fertility, such as the resumption of the estrous cycle, the ovulation of a competent oocyte, and the establishment and development of gestation [60]. Additionally, the temperature-humidity index calculated with the available meteorological information can be used to assess the effect of heat stress on animal welfare and embryonic survival.

Conclusions

Subclinical and inflammatory conditions of the recipients could be detected by using pathology clinical markers such as fibrinogen and hematological values to improve gestation outcomes in embryo transfer programs. Also, the criterion of discarding embryo recipients due to exposure to pathogens such as *B. abortus, N. caninum, and BVD* can be an efficient method in the control of vertically transmitted diseases limiting the risk of an embryo or fetal losses, and congenital or persistent infections that affect the health and reproductive efficiency of the herds. Consistently, a causal association between exposure to *BoHV-1* and L. *harjdo bovis and L canicola* serovars was observed leading to a decreased embryonic survival in embryo recipients, which highlights the importance of implementing adequate biosecurity programs that include vaccination, and pre-ET diagnostics. Similarly, this study was able to establish a positive effect of vaccination against reproductive pathogens on the pregnancy outcome following ET. From the genetic factors studied, it could be concluded that embryos from *Bos indicus* donors had a positive effect on the diagnosis of a viable gestation, and, that the Temperature-Humidity Index (ITH) is useful to define the environmental effect that affects embryo survival in an *in vitro* embryo transfer program.

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3. Conclusiones y recomendaciones

El éxito de los programas de transferencia embrionaria esta dado por la tasa de preñez, sin embargo los criterios de selección de receptoras también juegan un rol importante en dicho resultado. La presente investigación además evaluar la tasa de preñez, tuvo en cuenta la perdida embrionaria y aspectos cruciales para la selección de receptoras de embriones.

El examen minucioso y evaluación fisiológica de las receptoras de embriones cobra importancia, al evidenciar que alteraciones hematológicas y/o exposición a patógenos infecciosos reproductivos pueden causar perdidas embrionarias y fetales. Adicionalmente esta investigación evidencio la influencia de factores medioambientales y genéticos sobre la probabilidad de gestaciones viables en las receptoras de embriones in-vitro.

Los resultados de este trabajo contribuyen a tener una evidencia más amplia para la estructuración de programas de transferencia de embriones para considerar la relevancia de los criterios de selección de receptora, control de enfermedades reproductivas infecciosas, factores medioambientales, asociados con la receptora, factores instrinsecos/extrinecos, que además de favorecer la tasa de preñez, también favorecen la supervivencia embrionaria.