

Human chorionic gonadotropin a tool in the transfer of frozen embryos in double-purpose cows used as recipients

Gonadotrofina coriônica humana como instrumento na transferência de embriões congelados em vacas de duplo propósito utilizadas como receptoras

La gonadotropina corionica humana una herramienta en la transferencia de embriones congelados en vacas de doble propósito utilizadas como receptoras

DOI:10.34119/bjhrv7n3-375

Submitted: April 30th, 2024

Approved: May 21st, 2024

Netzi Naidí Méndez Palacios

Master in Veterinary Medicine and Animal Production by Universidad Autónoma de Puebla
Institution: Facultad de Medicina Veterinaria y Zootecnia BUAP
Address: El Salado, Tecamachalco, Puebla
E-mail: netzi.mendez@correo.buap.mx

Néstor Méndez Palacios

PhD in Sciences by Universidad Autónoma del Estado de México
Institution: Facultad de Medicina Veterinaria y Zootecnia BUAP
Address: El Salado, Tecamachalco, Puebla
E-mail: nestor.mendezp@correo.buap.mx

Felicitas Vázquez Flores

Master in Sciences by Universidad Nacional Autónoma de México
Institution: Facultad de Medicina Veterinaria y Zootecnia BUAP
Address: El Salado, Tecamachalco, Puebla
E-mail: felicitas.vazquez@correo.buap.mx

José Alfredo Galicia Domínguez

PhD in Eco-education by Instituto Universitario Puebla
Institution: Facultad de Medicina Veterinaria y Zootecnia BUAP
Address: El Salado, Tecamachalco, Puebla
E-mail: jose.galiciad@correo.buap.mx

Maximino Méndez Mendoza

PhD in Sciences by Universidad de Camagüey
Institution: Facultad de Medicina Veterinaria y Zootecnia BUAP
Address: El Salado, Tecamachalco, Puebla
E-mail: maximino.mendez@correo.buap.mx

ABSTRACT

The aim of this study was to evaluate the use of human chorionic gonadotropin at the time applied is performed embryo transfer (day 7) on pregnancy rate in embryo recipient cows in

dual purpose cattle, as recipients are used F1 cows (Holstein x Zebu and Zebu x Creole), totaling 300 cows cycling, regardless of the number of births. To synchronize, we used an ear implant containing 3 mg of progestogen norgestomet for 9 days, plus an intramuscular injection (IM) of 3 mg of norgestomet and 5 mg estradiol valerate. At implant removal, was applied 400 IU of equine chorionic gonadotropin (eCG) in both groups. The cows that showed oestrus, were transferred on day 7 of having submitted, only the cows were transferred to a rectal palpation had a corpus luteum of quality 2 and 3, using frozen embryos. The study was divided into two groups: group 1 (control with 70 cows) and group 2, treated with 1500 IU of eCG with 72 cows. Pregnancy diagnosis was performed at 60 days, the embryo transfer. The results were evaluated with a randomized design and statistical analysis, we used a Student t-test to determine the difference between the two groups. The results of this study show a significant difference ($P < 0.05$) in pregnancy rates, demonstrating that treatment with eCG at the embryo transfer increases pregnancy rates in cows transferred. Concluding that eCG treatment (1500 IU) resulted in higher pregnancy rate for the group treated 37 pregnant cows (51.3%) vs. the control group 29 cows pregnant (41.4%) ($P < 0.05$).

Keywords: human chorionic gonadotropin, reception, dual purpose cows, embryo transfers.

RESUMO

O objetivo deste trabalho foi avaliar o uso da gonadotrofina coriônica humana no momento em que é aplicada a transferência embrionária (dia 7) sobre a taxa de prenhez em vacas receptoras de embrião em bovinos de dupla finalidade, pois são utilizadas receptoras F1 (Holandês x Zebu e Zebu x Crioulo), totalizando 300 vacas ciclando, independente do número de partos. Para a sincronização, foi utilizado um implante auricular contendo 3 mg de progestágeno norgestomet por 9 dias, além de uma injeção intramuscular (IM) de 3 mg de norgestomet e 5 mg de valerato de estradiol. Na remoção do implante, foi aplicada 400 UI de gonadotrofina coriônica equina (eCG) em ambos os grupos. As vacas que apresentaram estro, foram transferidas no 7º dia após terem sido submetidas, apenas as vacas que foram transferidas à palpação rectal tinham um corpo lúteo de qualidade 2 e 3, utilizando embriões congelados. O estudo foi dividido em dois grupos: grupo 1 (controle com 70 vacas) e grupo 2, tratado com 1500 UI de eCG com 72 vacas. O diagnóstico de gestação foi realizado aos 60 dias, após a transferência dos embriões. Os resultados foram avaliados com um desenho aleatório e análise estatística, utilizamos um teste t de Student para determinar a diferença entre os dois grupos. Os resultados deste estudo mostram uma diferença significativa ($P < 0,05$) nas taxas de prenhez, demonstrando que o tratamento com eCG na transferência embrionária aumenta as taxas de prenhez nas vacas transferidas. Concluindo que o tratamento com eCG (1500 UI) resultou em maior taxa de prenhez para o grupo tratado 37 vacas prenhes (51,3%) vs. o grupo controle 29 vacas prenhes (41,4%) ($P < 0,05$).

Palavras-chave: gonadotropina coriônica humana, recepção, vacas de duplo propósito, transferência de embriões.

RESUMEN

El objetivo de este trabajo fue evaluar el uso de la Gonadotropina Coriónica Humana aplicada en el momento en que se realiza la transferencia de embriones (día 7) sobre el porcentaje de gestación en vacas receptoras de embriones en ganado bovino de doble propósito, como receptoras se utilizaron vacas F1 (Holstein x Cebú y Cebú x Criollo), con un total 300 vacas ciclando y sin considerar el número de partos. Para sincronizarlas, se utilizó un implante auricular que contenía un progestágeno 3 mg de Norgestomet, durante 9 días, más una inyección intramuscular (IM) de 3 mg de Norgestomet y 5 mg de valerato de estradiol. Al retiro del

implante, se aplicó 400 UI de Gonadotropina Coriónica equina (eCG), en ambos grupos. Las vacas que entraron en estro se transfirieron en el día 7 de haberlo presentado, solamente se transfirieron a las vacas que a la palpación por vía rectal presentaban un Cuerpo Lúteo de calidad 2 y 3; utilizando embriones congelados. El estudio se dividió en dos grupos; el grupo 1 (control con 70 vacas) y el grupo 2, tratado con 1500 UI de eCG con 72 vacas. El diagnóstico de gestación se realizó a los 60 días la transferencia de los embriones. Los resultados se evaluaron con un diseño completamente al azar y para el análisis estadístico, se utilizó una prueba de t-Student para determinar la diferencia entre los dos Grupos. Los resultados obtenidos en este estudio, muestran una diferencia significativa ($P < 0.05$) en los porcentajes de gestación, demostrando que el tratamiento con eCG al momento de transferir al embrión incrementa el porcentaje de gestación en las vacas transferidas. Concluyendo que el tratamiento con eCG (1500 UI), resultó en mayor porcentaje de gestación para el grupo tratado 37 vacas gestantes (51.3%) vs. el grupo testigo 29 vacas gestantes (41.4%) ($P < 0.05$).

Palabras clave: gonadotropina coriónica humana, vacas receptoras, vacas doble propósito, transferencia de embriones.

1 INTRODUCTION

Embryo transfer (ET) is a very important tool in genetic improvement; However, sometimes the results are affected by factors that influence the pregnancy percentages, among these are: the body condition of the recipients, heat stress (during the summer) that is capable of compromising the pregnancy (Al- Katanani *et al.*, 2001 , Méndez *et.*, 2022) number of births presented by both donor and recipient females and the nutrition of the recipients, as well as the follicular dynamics that can affect the percentage of pregnancy in the receiving cells (Baruselli, P. S. *et al.*, 2003), if there are nutritional or mineral deficiencies, the risk of embryonic death and a return to normal estrus increases; Embryonic maternal recognition is affected, as well as embryonic maternal synchrony.

Gonadotropin-releasing hormone (GnRH), used to prevent early embryo loss caused by CL lysis (Franco *et al.*, 2005), the use of Human Chorionic Gonadotropin (hCG) increases P4 levels, improving function of the corpus luteum (Changas and Lopes, 2004), preventing early regression of this structure from taking place. With hCG treatment, an increase in plasma P4 levels is observed and this is achieved by inducing the formation of accessory corpora lutea. Several methods have been used to increase pregnancy rates through plasma P4 concentrations during the luteal phase.

The objective of this work was to evaluate the use of eCG on day 7; moment in which the embryo transfer is performed, assessing conception percentages in dual-purpose cows used as recipients in the dry tropics.

2 MATERIAL AND METHODS

For the present work, 300 F1 animals (Holstein x Zebu and Zebu x Criollo) were used in group I (150 cows) in Group II (150 cows) they were selected based on their body condition (3.0 to 3.5), only They included cycling animals, supplemented with 4 kg of commercial feed with 18% crude protein, providing them at the time of milking 30 days before starting the transfer and 30 days after having transferred the embryo. As forage, they were offered cane tips and chopped sorghum. Both internal and external deworming was carried out using Ivermectins, as well as the application of Vitamins A and D and supplementation with mineral salts.

The experimental animals were palpated rectally to be able to evaluate their reproductive system, considering the presence of a corpus luteum (CL). 146 cows from group I and 146 cows from group II were synchronized as possible recipients. For this purpose, a auricular implant containing progestogen (3 mg of norgestomet) that remained in situ for 9 days in the pinna of the ear, plus a 2 ml injection containing 3 mg of norgestomet and 5 mg of estradiol valerate intramuscularly.

Once the 9 days have passed; The implant was removed and 400 IU of eCG or also called Pregnant Mare Serum Gonadotropin (PMSG) was applied intramuscularly to group II, which had the objective of recruiting a greater number of accessory LCs. Heat detection begins 24 hours after removing the implant for 3 days (day/night). The cows that came into heat were transferred on day 7 of having presented estrus; those cows that, upon palpation, presented a CL, manifest estrus and good body condition, were transferred. In this way, the cows were randomly assigned to group I and the same to group II.

Group 1 (n=73) was used as a control and group 2 (n= 73) was the experimental group and 1500 IU of hCG was applied intramuscularly at the time of embryo transfer.

Frozen embryos were transferred with ethylene glycol at morula or blastocyst stage from quality 1 (C1) to (C2). Defrosting was carried out in the following way: they were kept in air for 15 seconds and then another 15 to 20 seconds, in water at a temperature of between 25 and 27 °C. With the recipient epidurally anesthetized, the embryo was introduced into the uterus with an applicator in the last third of the uterine horn ipsilateral to the CL (previously palpated). After depositing the embryo in the uterine horn, 1500 IU of hCG per cow was applied.

The results were evaluated according to the percentage of pregnancy that occurred in each group (the diagnosis was made through rectal palpation 60 days after transfer).

2.1 VARIABLES EVALUATED

- a) Presentation of estrus; treated and untreated cows
- b) Quality of CL 1, 2, 3; treated and untreated for transfer
- c) Percentage of treated and untreated pregnancy in transferred cows.

3 RESULTS

The control group (Group I) 112/146 females presented estrus, this is equivalent to 76.7%, in the treated group (Group II) 110/146 females presented estrus, representing 75.3% of synchronized females showing a non-significant difference ($P>0.05$). The females that presented estrus were checked on day 6 post-estrus by means of rectal palpation in order to find corpora lutea of good quality, to be transferred, for which they were classified on a scale from 1 to 3 (1 = poor, 2 = good, 3= excellent), those CL with quality 2 and 3 being better; Therefore, group I was selected for transfer (n=70 animals) presenting quality of CL2= 39/70 (55.7%) and CL3= 31/70 (44.2%), in group II (n=72 animals) CL2= 43/72 (40.2%) and CL3 = 29/72 (59.8%).

At the time of transfer (day 7), Group I received an injection of Physiological Saline Solution (FSS) and Group II received a dose of 1500 IU of hCG. After this, 60 days after the transfer, the pregnancy diagnosis was made in the transferred animals, and it was observed that in Group I; 29/70 (41.4%) females were pregnant and 41/70 (58.6%) were not pregnant; Of these, 16/29 had CL2 (55.2%) and 13/31 with CL3 (44.8%). While in Group II, 37/72 females (51.4%) were diagnosed as pregnant and 35/72 (48.6%) were found to be non-pregnant; of these 18/37 with CL2 (48.6%), 19/37 with CL3 quality (51.4%), showing no significant difference ($P>0.05$) in CL2 quality and having a significant difference ($P<0.05$) in CL3 quality after the transference.

4 DISCUSSION

In this work it was found that of the 292 females synchronized with a synthetic progestogen (Norgestomet), 76.7% and 75.3% of group I and II respectively presented estrus. These results do not show a significant difference ($P>0.05$) between the treatments. Mata *et al.* (2001) obtained Mata 75% synchronization using Norgesomet, this is similar to what was found in this work. In relation to the quality of the Corpus Luteum, in the cows that were palpated

rectally and selected for embryo transfer. Group I presented 55.8% CL2 and Group II 59.7% CL2, obtaining a significant difference ($P < 0.05$) in quality of CL, while in Quality of CL3; in Group I it was 44.8% and Group II 40.3%, there was no significant difference ($P > 0.05$), it is considered important to establish the size and consistency of the CL, that is, that it present good to excellent quality. Nishigai et al., (2001) consider it as an associated factor for the selection of recipients for embryo transfer. Regarding the percentage of pregnancy in the transferred females, it was observed that in Group I (41.4%) and Group II (51.3%) they were pregnant, finding a significant difference ($P < 0.05$), the results obtained indicate that when applying hCG in the day 7 post-estrus in recipient females, increases pregnancy percentages; These results are lower than those reported by (Nishigai et al., 2002) who obtained 67%, when administering 1500 IU/cow of hCG to reinforce luteal function on day 6 post-estrus in recipients of (frozen) embryos.

In conclusion, the results obtained in this study indicate that treatment with hCG at the time of embryo transfer increases the pregnancy percentage in recipient cows.

REFERENCES

1. Al-Katanani Y M, Drost M, Monson R L, Rutledge J J, Krininger C EIII, Block J. 2001. Pregnancy rates following timed embryo transfer with fresh or vitrified in vitro produced embryos in lactating dairy cows under heat stress conditions. *Theriogenology* 58: 171-182.
2. Baruselli, P. S., Marques, M. O., Carvalho, N. A. T., Valentim, R., Berber, R. C. A., Carvalho Filho, A. F., Madureira, E. H., and Costa Neto, W. P. 2003. Follicular dynamics and pregnancy rate in embryo recipient (*Bos taurus indicus* × *Bos taurus taurus*) treated with Ovsynch protocol for fixed-time embryo transfer. *Brazilian Journal of Veterinary Research and Animal Science*, 40: 96-106
3. Changas E S J, Lopes Da C L. 2004. Luteotrophic influence of early bovine embryos and the relationship between plasma progesterone concentrations and embryo survival. *Theriogenology* 64 (1): 49-60.
4. Franco M, Block J, Jousan F D, De castro e Paulo L A, Brad A M, Franco J M. 2005. Effect of transfer of one or two in vitro-produced embryos and post-transfer administration of gonadotropin releasing hormone on pregnancy rates of heat-stressed dairy cattle. *Journal of Animal Science* 66: 224-233.
5. Mata Castellanos F A, Hernandez C J, Gonzalez P E. 2001. Efecto del norgestomet inyectado sobre el folículo dominante persistente y la formación del cuerpo luteo en vacas sincronizadas con implantes de Norgestomet. *Vet. Mex.* 32: 19-25.
6. Méndez PN, Flores VF, Cordova IA y Méndez MM. 2022 Distúrbios reprodutivos em bovinos leiteiros causados por estresse térmico. Trastornos reproductivos en bovinos lecheros causados por estrés calórico. *Brazilian Journal of Animal and Environmental Research* 1336 - 1341. ISSN: 2595-573X DOI: 10.34188/bjaerv5n1-103
7. Nishigai M, Kamomae H, Tanaka T, Kaned Y. 2002. Improvement of pregnancy rate in Japanese Black cows by administration of hCG to recipients of transferred Frozen-thawed embryos. *Theriogenology* 58: 1597-1606.
8. Nishigai M, Takamura A, Kamomae H, Tanaka T, Kaneda Y. 2001. The Effect of Human Chorionic Gonadotropin on the Development and Function of Bovine Corpus Luteum. *Journal of Reproduction* 47: 283- 294.