Article **Journal of Natural and Agricultural Sciences**

December 2023, Vol.10 No.27 22-26

Use of rapid test glycoproteins associated with pregnancy and ultrasound in early diagnosis of pregnancy in cow

Uso de la prueba de glucoproteínas asociadas a la gestación y la ecografía en el diagnóstico precoz de la gestación en bovinos productores de leche

GARCÍA-GOZÁLEZ, Daniela, ALARCÓN-ZAPATA, Marco Antonio, GARCEZ-MERCADO, Nora and TABAREZ-ROJAS, Abigail*

Facultad de Ciencias Biológicas y Agropecuarias, Universidad Veracruzana. Tuxpan, México. Facultad de Ciencias Biológicas y Agropecuarias de la Universidad Veracruzana. Carretera Tuxpan-Tampico Km. 7.5, Colonia Universitaria, Tuxpan, Veracruz, México, CP 92895. Tel. 017838344350 ext. 46109.

ID 1st Author: *Daniela, García-González* / **ORC ID** 0009-0009-6590-6587

ID 1st Co-author: *Nora, Garcez-Mercado* / **ORC ID** 0000-0002-4712-4663

ID 2nd Co-author: *Marco Antonio, Alarcón-Zapata* / **ORC ID** 0000-0002-4712-6327, **CVU CONAHCYT ID**: 176712

ID 3rd Co-author: *Abigail, Tabarez-Rojas* / **ORC ID** 0000-0002-8766-6993, **CVU CONAHCYT ID**: 176667

DOI: 10.35429/JNAS.2023.27.10.22.26 Received July 25, 2023; Accepted December 30, 2023

Abstract

The study was carried out with the objective of determining the efficiency of pregnancy-associated glycoprotein test compared to ultrasound. 146 cows of the Holstein Friesian breed were used. The early diagnosis of pregnancy was made with the ELISA test at 28 days post-breeding and the first confirmation by ultrasound on day 35 and day 50 post-breeding a second confirmation. Statistical analysis was performed with chi-square test to determine the differences between the diagnostic test (rapid test and ultrasound). Differences were observed (P=0.009) between the two diagnostic tests, 68.5% of pregnant animals were detected with the blood test and 47.3% with ultrasound, with respect to ultrasound 1 and 2, no statistically significant differences were observed (P=0.1). The blood test allows the early diagnosis of cows that were not pregnant, and, in this way, they can be reincorporated into reproductive management practices, reducing the period of days open.

Glycoproteins associated with pregnancy, Ultrasound, Cow

Resumen

El estudio se realizó con el objetivo de determinar la eficiencia de la prueba de glucoproteínas asociadas a la gestación en comparación a la ecografía. Se utilizaron 146 vacas de la raza Holstein Friesian. Se realizó el diagnóstico precoz de gestación con la prueba ELISA a los 28 días post-servicio y la primera confirmación mediante la ecografía al día 35 y posteriormente al día 50 una segunda confirmación. El análisis estadístico se realizó con la prueba chi cuadrada para conocer las diferencias entre las pruebas diagnósticas (Prueba rápida y Ecografía). Se observaron diferencias (P=0.009) entre las dos pruebas diagnósticas, se detectó el 68.5% de animales gestantes con la prueba en sangre y 47.3% con la ecografía, respecto a la ecografía 1 y 2 no se observaron diferencias estadísticamente significativas (P=0.1). La prueba en sangre permite el diagnóstico temprano de vacas que no quedaron gestantes y de esta manera pueden ser reincorporadas a las prácticas de manejo reproductivo, reduciendo el periodo de días abiertos.

Glucoproteínas asociadas a la gestación, Ecografía, Vacas

Citation: GARCÍA-GOZÁLEZ, Daniela, ALARCÓN-ZAPATA, Marco Antonio, GARCEZ-MERCADO, Nora and TABAREZ-ROJAS, Abigail. Use of rapid test glycoproteins associated with pregnancy and ultrasound in early diagnosis of pregnancy in cow. Journal of Natural and Agricultural Sciences. 2023. 10-27:22-26.

* Author Correspondence (Email: atabarez@uv.mx)
† Researcher contributing as first author.

Introduction

For years reproduction has been an important factor for large dairy producers, with new technologies in the dairy industry the greatest objective is to take advantage of all types of opportunities, animals producing high quantities of milk and producing offspring, as well as reducing the parameter open days and economic losses (Lee, 2007). Currently, the reduction of open days in a dairy herd constitutes one of the biggest challenges for veterinary technicians or doctors in order to obtain high reproduction parameters.

The duration of gestation is 283 days (243-316 days) and can be divided into an embryonic period, which runs from fertilization to 45 days, and a fetal period, from 46 days to delivery. The duration of gestation is influenced by maternal, fetal, genetic and environmental factors (Hernández, 2016).

One of the great obstacles for large production units are abortions, which is the expulsion of a fetus with some pathology, sometimes immature (premature). From 20 to 25 percent of cows diagnosed as pregnant lose pregnancies after day 45 of service. Like embryonic death, if it dies in the first 18 days of pregnancy it is considered an early embryonic death: when this happens, it can be observed that the cow presents estrus from days 21 to 24 after breeding (Wiltbank *et al.*, 2016).

If the embryo dies between days 24 to 42 (before organogenesis is completed), it is considered a late embryonic death; when this happens in cows, intrauterine resorption of the embryo occurs and a delay in the appearance of estrus is observed. But if the death of the embryo occurs after day 45, it is considered fetal death (Diskin and Morris, 2008; Hernández, 2016).

When the embryos or fetuses die in these cases, they are very small, and this goes unnoticed in the pens due to the size in which they are found and, in these situations, the cows only return to estrus. Producers limit themselves to days 28 and 30 of gestation as the closest point for accurate diagnosis of pregnancy due to the effectiveness of ultrasound and chemical methods using pregnancy-associated glycoproteins that are commercially available.

Interferon-tau (IFNτ) is taken as a sign of early pregnancy since it prevents luteolysis of the corpus luteum (CL), by directly or indirectly blocking the synthesis of oxytocin receptors in the endometrium and consequently the pulsatile production of PGF2α (Hernández, 2018).

The detection of pregnancy-associated proteins (PAG) using ELISA is considered a sensitive and accurate alternative for the diagnosis of early pregnancy in cattle, in addition to being non-invasive, therefore the objective of the study was to compare the efficiency of the test for proteins associated with pregnancy and ultrasound in the early diagnosis of pregnancy in dairy-producing cattle.

Material y methods

Location of the study area

This study was carried out on a farm in Actopan, Hidalgo, at an altitude of 2260 meters above sea level, with a semi-dry temperate climate, its minimum temperature ranges between 5.3°C reaching a maximum of 24.4°C (INEGI, 2017).

Experimental design

Multiparous Holstein Friesian breed cows (n=146) were used. The cows were stabled and milked twice a day. Pregnancy diagnosis was made in all cows by means of the rapid visual test for detection of glycoproteins associated with pregnancy in blood serum and by rectal ultrasound.

To perform the rapid visual test and detect pregnancy-associated glycoproteins (PAG), on day 28 post-breeding, a blood sample (approximately 8 ml) was taken from each cow by puncture of the coccygeal vein, using a caliber needle 21g and a vacutainer tube without anticoagulant. The blood samples were centrifuged at 3900 rpm for 5 minutes to obtain the blood serum and process it by enzyme immunoassay (ELISA) with the Bovine Pregnancy Test from the IDEXX laboratory, following the manufacturer's instructions. The presence of PAG was determined when the samples turned blue like the positive control.

ISSN: 2410-356X

Pregnancy diagnosis by ultrasound was performed on all cows at 35±7 days post-breeding and was reconfirmed 15 days later. A Chison ECOVET-2 portable ultrasound machine with a 6 MHz real-time linear transducer was used. A female was considered pregnant when the amniotic vesicle was visualized, and the embryo's heartbeat was detected.

Statistical analysis

Data were analyzed with the Chi-square statistical test using the SPSS® 20 suite for Windows® (IBM SPSS, 2011).

Results

Of the 146 cows served, 100 pregnant cows were detected by the rapid test and 69 pregnant cows by the first ultrasound (figure 1), finding a significant difference for the diagnosis of pregnant cows (P=0.009).

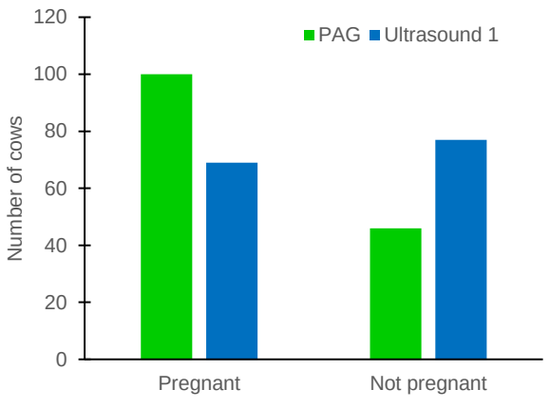

Figure 1 Diagnosis of pregnancy in cows using the rapid pregnancy-associated glycoprotein (PAG) test and first ultrasound.

The results obtained in the diagnosis of pregnancy through ultrasound in the second confirmation were maintained in 69 pregnant cows (Figure 2), which indicates that there were 31% embryonic losses in the period 35±7 days post-breeding according to the results obtained with the glycoprotein test associated with pregnancy in the period of 50±7 days post-breeding, without finding differences (P=0.1) in the diagnosis of pregnancy between ultrasound 1 and ultrasound 2, this means, that ultrasound is 21% more effective compared to the rapid test for glycoproteins associated with pregnancy, the latter being 69% effective.

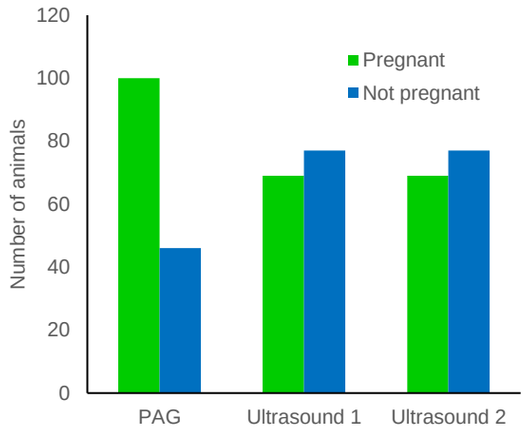

Figure 2 Number of pregnant cows according to the rapid pregnancy-associated glycoprotein (PAG) test, first ultrasound and confirmation ultrasound.

Table 1 shows the percentages obtained through the rapid pregnancy diagnosis test and ultrasound; for false positives, 21.24% and 0% were obtained respectively. The true positives for the pregnancy diagnosis were 69 animals, the ultrasound was 100% effective in determining pregnancy in the cows, however, with the rapid test there were 31 false positive animals that were considered embryonic losses because at the time of performing the test were pregnant, however, when the ultrasound was performed, they were no longer pregnant.

As for the true negatives for pregnancy, there were 46 animals for the rapid test and 77 animals for the ultrasound.

Diagnostic	Rapid test		Ultrasound	
	(n)	(%)	(n)	%
False positives	31	21.2	0	0
False negatives	0	0	0	0
True positives	69	47.3	69	47.3
True negatives	46	31.5	77	52.7
Total	146	100	146	100

Table 1 Percentage of false positives, false negatives, true positives and true negatives, through the rapid pregnancy-associated glycoprotein (PAG) test and ultrasound

ISSN: 2410-356X

Discussion

The percentage of pregnancy with the rapid test for glycoproteins associated with gestation at 28 days post-breeding was 68.5%, while with ultrasound at 35±7 days and 50±7 days post-breeding, 47.3% were detected. of pregnant animals, there being a difference of 21.2% between both methods, which contrasts with what was observed by Hernández (2020), in cows of dairy breeds when carrying out the pregnancy diagnosis with both the ELISA IDEXX kit and with ultrasound, they determined that both methods are closely related because there is no variation between the results of the diagnostic tests. Similarly, Antelo and Ibáñez (2015), in beef breed cows when carrying out the diagnosis of pregnancy with the IDEXX rapid test and rectal palpation, did not find statistically significant differences in the percentage of pregnancy between both tests, on the contrary, Garate and Suárez (2015), when comparing the IDEXX rapid test at 28 days post-breeding and rectal palpation at 60 days post-breeding in milk-producing cows, determined a minimal difference between the diagnostic methods, determining 47.6% of gestation for the ELISA test and 40.7% for rectal palpation, pointing out that the ELISA method with the IDEXX kit is very precise and that the difference between diagnostic methods may be affected by embryonic death or skill of the personnel at the time of perform the diagnosis.

In this sense, in this study it was considered that the difference in the pregnancy results obtained between the methods for diagnosing pregnancy was due to embryonic losses (31%), since it is well known that during the first 60 days of pregnancy losses occur (Sice *et al.*, 2022) and in the event that there is an embryonic loss, the proteins associated with pregnancy remain circulating in the blood for 7 to 14 days after the loss (Humbolt, 2002), the half-life of trophoblastic proteins varies depending on the degree of glycosylation they present in their structure, although on average it is described as 7.2 days (Jerome, 2012). On the contrary, ultrasound is more accurate in detecting pregnancy by identifying the amniotic vesicle and heartbeat. However, as Garate and Suárez (2015) point out, the IDEXX kit helps identify empty cows early, allowing them to be reintegrated into new synchronization protocols, saving time and money.

Another aspect to consider in the results is the percentage of embryonic loss in the study (31%), this result is below what Diskin *et al*. (2012) reported for high-producing cows, the rate of embryonic and fetal loss ranges from 40 to 56% respectively. However, improving the embryonic survival rate remains the main challenge, given the antagonistic relationship between embryo production and survival.

Conclusion

The use of the protein test for early detection of pregnancy at 28 days post-breeding makes it possible to determine pregnant cows with 100% efficiency, helping to detect cows that are empty very early to reintegrate them into new protocols synchronization and/or artificial insemination or natural mating, reducing the interval of days open.

References

Antelo, BVA. & Ibáñez, DRLA. (2015). Detección temprana de preñez utilizando el kit IDEXX Bovine pregnancy en vacas de carne. Tesis de licenciatura. Escuela Agrícola Panamericana, Zamorano, Honduras.

Diskin M. & Morris, D. (2008). Embryonic and early fetal losses in cattle and other ruminants. Reprod domest anim 43:260–267.

Diskin, M., Parr, M. & Morris, D. (2012). Embryonic death in cattle: An update. Reproduction, Fertility and Development 24:244–251.

Garate, W. & Suárez, W. (2015). Diagnóstico temprano de preñez con prueba Elisa en ganado lechero mediante el kit IDEXX Bovine Pregnancy, Tesis de licenciatura. Escuela Agrícola Panamericana, Zamorano, Honduras.

Hernández, CJ. (2016). Fisiología clínica de la reproducción de bovinos lecheros. UNAM.

Hernández M.J.H. 2018. Capítulo 10: Establecimiento de la gestación. En: Rangel P.L. y Hernández M.J.H. Fisiología reproductiva de los animales domésticos. UNAM. Pag. 171-187.

ISSN: 2410-356X

Hernández, SJA. (2020). Comparación del resultado de la prueba ELISA IDEXX rapid pregnancy test en vacas gestantes, con ecografía, en la hacienda Santa Clara de la provincia del Carchi, Ecuador. Tesis de Licenciatura. Facultad de Ciencias de la Salud. Ecuador.

Humbolt, P. (2002). Use of pregnancy specific proteins and progesterone assays to monitor pregnancy and determine the timing, frequencies and sources of embryonic mortality in ruminants. Theriogenology 56:1417-1433.

IBM, Corp. Released. (2011). IBM SPSS Statistics for Windows, Versión 20.0. Armonk, NY; IBM Corp.

Instituto Nacional de Estadística y Geografía (INEGI). (2017). Anuario estadístico y geográfico de Hidalgo, México. 671p.

Jerome, A. (2012). An overview on Pregnancy Associated Glycoproteins in Cattle and Buffalo. J Adv Vet Res 2: 50-58.

Lee, J. (2007). Pregnancy loss in dairy cows: the contributing factors, the effects on reproductive performance and the economic impact. J Vet Sci 8: 283e8...297.

Sice, M., Gómez-Martín, A. & Gomis, J. (2022). Presente y futuro del diagnóstico de gestación en el ganado bovino. AN. VET. (MURCIA) 36: 1-18. DOI: 10.6018/analesvet.503631

Wiltbank, MC., Baez, GM, Garcia-Guerra, A., Toledo, MZ., Monteiro, PL. & Melo, LF. (2016). Pivotal periods for pregnancy loss during the first trimester of gestation in lactating dairy cows. Theriogenology 86:239e53.

ISSN: 2410-356X