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EFFICACY OF PREGNANCY DIAGNOSTIC METHODS IN THE REPRODUCTIVE PERFORMANCE OF CATTLE: A META-ANALYSIS

Eficiencia de los métodos de diagnóstico de la preñez en el desempeño reproductivo de bovinos: Meta-análisis

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ABSTRACT

Traditional early pregnancy diagnostic methods are rectal palpation (RP) or transrectal ultrasonography (TRUS) before 35-45 days post AI. There are alternative tests such as tests for progesterone (P4), pregnancy-associated glycoprotein (PAG) and interferon-stimulated genes (ISG) have been developed. One statical approach was conducted using random effects meta-analysis including four methods of early pregnancy detection. A systematic review of sources such as PubMed, Google Scholar and Scielo was carried out, with the aim to determine the most accurate and similar pregnancy diagnosis method compared to rectal palpation or ultrasound (gold standard). One statical approach was conducted using random effects meta-analysis including four methods of early pregnancy detection. A reproductive outcome of interest was the diagnostic accuracy of pregnant cows for each method. All the results show us odds ratio (OR) greater than 1, which means that there is a higher probability of detecting pregnant cows using these methodologies instead of the gold standard. Although, the only method with a significant result ($p<0.05$) is the P4 test. Since the OR value exceeds 1, it means that the evaluated method detects pregnant cows with more precision compared to the gold standard. This numerical difference would mean that all animals initially diagnosed as positive (pregnant cows) either suffered embryonic/foetal mortality or were diagnosed

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as false positives. Otherwise, when the OR value is closer to 1, it means that the diagnostic accuracy of pregnant cows will be maintained until the second diagnostic method (gold standard). In this study, the TRUS test was closest to 1. In conclusion, pregnancy detection by the progesterone method is associated with high rates of false positives or embryo mortality. The TRUS method is the method that has the greatest similarity to the gold standard when it comes to pregnant cows, followed by PAG and ISG.

Keywords: pregnancy diagnostic, cattle, reproductive performance, ISG-PAG-TRUS, odds ratio.

RESUMEN

Los métodos de diagnóstico de preñez temprana tradicionales son la palpación rectal (PR) o la ultrasonografía transrectal (TRUS) antes de los días 35-45 post IA. Existen pruebas alternativas como la prueba de progesterona (P4), la glicoproteína asociada a la preñez (PAG) y los genes estimulados por interferón (ISG). Con el objetivo de determinar el método de diagnóstico de preñez con más precisión y con mayor similitud en comparación con la palpación rectal o ultrasonido (gold standard). Se realizó un enfoque estático utilizando un meta-análisis de efectos aleatorios que incluía cuatro métodos de detección temprana de gestación. Un resultado reproductivo de interés fue la precisión de diagnóstico de vacas preñadas para cada método. Todos los resultados nos muestran relación de probabilidades (OR) superior a 1, lo que significa que existe una mayor probabilidad de detectar vacas preñadas utilizando estas metodologías en lugar del estándar de oro. Aunque, el único método con un resultado significativo ($p<0,05$) es la prueba P4. Como el valor de OR supera 1, significa que el método evaluado detecta con más precisiones vacas preñadas en comparación con el patrón oro. Esta diferencia numérica significaría que todos los animales diagnosticados inicialmente como positivos (vacas gestantes) o sufrieron mortalidad embrionaria/fetal o fueron diagnosticados como falsos positivos. De lo contrario, cuando el valor de OR esté más cerca de 1, significa que la precisión de diagnóstico de las vacas preñadas se mantendrá hasta el segundo método de diagnóstico (estándar de oro). En este estudio, la prueba TRUS fue la más cercana a 1. En conclusión, la detección del embarazo por el método de la progesterona está relacionada con altas tasas de falsos positivos o mortalidad embrionaria. El método TRUS es el método que tiene mayor similitud con el patrón oro cuando se trata de vacas preñadas, seguido por PAG e ISG.

Palabras clave: preñez, ganado, desempeño reproductivo, ISG-PAG-TRUS, razón de probabilidades

1. INTRODUCTION

An early and accurate pregnancy diagnosis is an essential activity for the reproductive management of cattle, sheep, horses, etc. Identifying empty (non-pregnant) females allows the implementation of synchronization and reproductive management programs that allow an early effective service (Shephard and Morto, 2018). In dairy cattle, keeping in an open physiological state generates substantial economic losses for the farmer. French and Nebel (2003) estimated that for a period of empty days greater than 175 after calving, the economic loss amounts to 4.95 dollars per day, this for intensive breeding of Holstein cattle in the United

States. Likewise, Diskin and Morris (2008) demonstrated an increase in the pattern of early embryonic death in high-production Holstein cows, which directly affects the increase in the period of empty days.

Rectal palpation (RP) is the most widely used method of pregnancy diagnosis in recent decades in cattle. However, the accuracy of this method of pregnancy diagnosis depends on the ability to recognize changes in the tone, size, and location of the uterine horns, as well as changes in the uterine arteries (Gunn and Hal, 2018). Experienced probes can achieve 95 to 99% accuracy by performing tests between days 45 to 120 of the gestation stage. On the other hand, currently, real-time ultrasound has become the method of choice for early pregnancy diagnosis by many modern veterinarians and producers. The search for other methods of diagnosing pregnancy earlier in a repeatable way provides the breeder with an option to incorporate cows into the production cycle and reducing the interval between calving (Ealy and Seekford, 2019). Some of these methods are progesterone levels in blood or milk, glycoprotein tests associated with pregnancy and Interferon stimulated genes (ISG). The ISG can be detected on circulating peripheral mononuclear bold cells by using quantitative reverse transcription PCR at day 17 to 22 of pregnancy in heifers and cows (Ealy and Seekford. 2019). Pregnant animals tend to have increased expressions of ISG15, MX1, MX2 and OAS-1 (Speckhart et al., 2018). Progesterone tests (P4) are commercially available and performed between day 20 and 24 post-insemination, can correctly diagnose pregnancy in 60% to 100% of cows (Ealy and Seekford, 2019).

PAG tests can be performed between day 24 and 30 of gestation accurately (Reese et al., 2018), since after day 35 post insemination the use of transrectal ultrasound is more accurate, fast, and accurate, but requires trained personnel for its use (Fricke et al., 2016).

Selecting an early pregnancy diagnostic technique as a practical, economical, and accurate tool will help manage reproduction in a cattle herd efficiently. Therefore, a meta-analysis comparing various methods of diagnosing early pregnancy using statistical techniques that combine results from a systematic review of the scientific literature would allow to synthesize, analyze, and recommend the most appropriate. Our objectives are determining the method that detects the largest number of pregnant cows and determining the pregnancy method with the greatest similarity in the number of pregnant cows compared to rectal palpation or ultrasound (gold standard).

2. MATERIALS AND METHODS

Literature Search

Relevant literature was identified through a comprehensive search of PubMed, Google Scholar and Scielo. The data selected was between 1995 and 2020. Search terms included are: “cattle”, “pregnancy diagnosis”, “rectal palpation”, “ultrasound”, “early pregnancy diagnosis”, “PAG” and “ISG”. More than 250 articles were identified and were further examined to determine suitability for inclusion utilizing PRISMA guidelines for systematic reviews. Primary screening was based on title and abstract information to establish whether in the article there was a reporting on reproductive performance in cattle. Articles meeting these criteria were further evaluated for data extraction.

Inclusion Criteria and Exclusion Criteria

We consider only randomized studies using studies that analyze techniques for diagnosing pregnancy in cattle in the context of reproductive performance, which report specificity and sensitivity over 56% or similar values as part of the manuscript or supplementary data. Other mandatory inclusion criteria included studies that used reproductive performance as the endpoint of reproductive efficiency of cattle with at least two treatments: gold standard and the treatment to be evaluated. Hence, conference abstracts, reviews or letters to the editor were not included or incomplete thesis and studies with a sample size of less than 15 bovine were excluded. Also, studies that report in vitro studies or do not report early pregnancy diagnostic techniques were excluded as part of reproductive efficiency. In addition, studies with pregnancy diagnosis after days 60 of pregnancy or that included trials with bias pregnancy success, such as induced twinning were excluded from the meta-analysis.

Based on these criteria, the meta-analysis included four methods of early pregnancy detection: ISG (Interferon Stimulated Genes) from 15 to 22 days post insemination, PAG (Pregnancy Associated Glycoprotein) from 25 to 30 days, P4 (Progesterone) from 15 to 25 days and TRUS (Transrectal Ultrasonography) between 25 to 35 days.
Articles were also selected based on the method used as gold standard. For instance, in the first three methodologies the gold standard used was transrectal ultrasonography in the range of day 25 to 30 post insemination. Meanwhile, evaluating the progesterone methodology and transrectal ultrasonography, the gold standard was rectal palpation between 40- and 60-days post insemination.

Data Extraction

Data extraction was performed by a single investigator. For each study recorded information included authors, year of publication, country, number of animals, breed, category, physiological state, type of management, samples, pregnancy rate, days of sample taken, sensibility, specificity, type of method, gold standard, days of sample taken for the gold standard and pregnancy loss. Within the study, sensitivity was calculated as the number of cattle correctly diagnosed with a positive pregnancy test / the number of cattle with a positive gold standard pregnancy test x 100. Specificity was calculated as the number of cattle correctly diagnosed with a negative pregnancy test / number of cattle with a negative gold standard pregnancy test x 100.

Statical Analysis

The meta-analysis was conducted using IBM SBSS Statics program version 28.0. The binary method was used with a random effects model with raw data, where the admission data is Treatment (success and fail) and Control (success and fail).

The meta-analysis was executed from 37 data from 34 articles, which were subdivided in 4 subgroups: ISG, PAG,P4 and TRUS evaluating the reproductive performance based on pregnancy detection (Melo et al., 2020; Han et al., 2006; Yoshino et al., 2020; Yoshino et al., 2018; Wijma et al., 2016; Serrano et al.,2020; Pugliesi et al., 2014; Soumaya et al.,2016 Dufour et al., 2017;Karen et al., 2015; Kaya et al., 2016; Mayo et al., 2016; Meziane et al., 2021; Moussafir et al., 2018; Piechotta et al., 2011; Shephard et al., 2018; Silva et al., 2007; Green et al., 2009; Filho et al., 2020; Commun et al., 2016; Sinedino et al., 2014; Romano et al., 2010; Holtz et al., 2019; Lee et al., 1996; Wu et al., 2014; Faustini et al., 2007; Abdulla et al., 2014; Chaudhary et al., 2012; Nation et al., 2003; Quintela et al., 2012; Romano et al., 2006; Scenzi et al., 1999; Scenzi et al., 1995; Scully et al., 2014).

To measure the size of the effect, it should be considered that the type of data included, binary data, and the type of effect size were evaluated using Odds ratio. In this case, the Odds ratio is a relationship between the possibility of detecting cows pregnant by the methodology and the possibility of detecting pregnant cows by the gold standard.

$$OR = \frac{(FP+TN)/(TP+FN)}{(FN+TN)/(TP+FP)} = \frac{\textit{Possibility of detecting cows pregnant using the methodogly in question}}{\textit{Possibility of detecting pregnant cows by the gold standard}}$$

Where TP true positive, FP is false positive, FN represents false negative, and TN is true negative.

If OR is 1, the possibility of not becoming pregnant is equal when performing pregnancy detection by the gold standard or through the treatment to be evaluated. There is an absence of association.

If OR >1 there is a greater possibility of detecting pregnant cows using the treatment to be evaluated. There is a positive association.

If OR <1 there is a greater chance of detecting pregnant cows using the gold standard. There is a negative association.

The heterogeneity tells us how much one study varies with respect to the other. In this study, heterogeneity was evaluated by two methods:

- The overlap of the 95% Confidence Intervals (CI) with the estimated line determines that there is no heterogeneity. The more distant the studies, it is a clear sign of high heterogeneity.
- Index of inconsistency of the studies. Where the values of 25%, 50% and 75% in the I^2 test correspond to low, medium, and high levels of heterogeneity, respectively. It is calculated as follows: I2= 100% (Q – df)/Q, where Q is Cochran's heterogeneity static and df is the degrees of freedom. To correctly interpret the I^2 statistic, the following ranges of values will be used (Higgins and Green, 2011): 0%-40% might not be important; 30%-60% may represent moderate heterogeneity; 50%-90% may represent significant heterogeneity and 75%-100% represents considerable heterogeneity.

3. RESULTS

The results of the summary of the variable analyzed pregnancy detection found based on positive pregnancy detection considered as "success" and not pregnant considered as "fail" are presented in Table 1. According to these variables, it could be calculated the sensibility and specificity for each method. For instance, the articles selected for ISG method have a sensibility that goes from 67% to 99% and their specificity from 62% to 90%. The PAG method goes from 74% to 100% and 88% to 100% respectively. P4 method goes from 82% to 98% and 68% to 96%. Finally, ultrasound goes from 92% to 100% and 63% to 98%.

On average, ISG is used at day 20, PAG at day 28, P4 at day 21 and TRUS at day 26. And for the Gold standard, for RP was 55 days while using TRUS, the average day of test was 35 post insemination (Table 1).

Our objective is to analyze which method is more accurate to detect pregnant cows between ISG, PAG, P4 and TRUS methodologies versus the gold standard, which in this case are TRUS and RP.

According to Table 2, in all the subgroups (ISG, PAG, P4 and TRUS) the OR is over 1, meaning that there is a greater chance of detecting pregnant cow using these

Table 1. Period and classification of the studies.

Reference	Country	Treatment		Control		Treatment		Gold standard	
		Succes	Fail	Succes	Fail	Type[1]	Days	Type[2]	Days
Melo et al., 2020	BRA	68	76	84	60	ISG	20	DOPPLER	20
Han et al., 2006	USA	34	44	26	52	ISG	18	TRUS	32
Yoshino et al., 2020	JPN	60	37	50	47	ISG	21	TRUS	30
Yoshino et al., 2018	JPN	27	30	28	29	ISG	21	TRUS	30
Wijma et al., 2016	USA	37	26	26	37	ISG	20	TRUS	42
Serrano et al.,2020	SPN	36	17	35	18	ISG	20	TRUS	37
Pugliesi et al., 2014	BRA	29	61	33	57	ISG	20	TRUS	30
Soumaya et al.,2016	IND	9	6	9	6	ISG	20	TRUS	18
Dufour et al., 2017	CAN	276	221	257	240	PAG	28-45	TRUS	28-45
Karen et al., 2015	HUN	38	62	41	59	PAG	28	TRUS	28
Kaya et al., 2016	TUR	21	30	22	29	PAG	28	TRUS	30
Mayo et al., 2016	USA	164	156	159	161	PAG	28	TRUS	38
Mayo et al., 2016	USA	161	159	159	161	PAG	28	TRUS	38
Meziane et al., 2021	DZA	26	15	25	16	PAG	28	TRUS	33
Moussafir et al., 2018	MAR	140	53	128	65	PAG	30-40	TRUS	45
Piechotta et al., 2011	DEU	149	36	151	34	PAG	26-58	TRUS	26-58
Shephard et al., 2018	NZL	501	378	458	421	PAG	70-100	TRUS	85-115
Silva et al., 2007	USA	409	473	379	503	PAG	27	TRUS	32
Green et al., 2009	USA	283	329	257	355	PAG	25-45	TRUS	25-45
Filho et al., 2020	USA	298	379	349	328	PAG	24	TRUS	30
Commun et al., 2016	FRA	68	34	63	39	PAG	30	TRUS	41
Commun et al., 2016	FRA	66	36	63	39	PAG	30	TRUS	41
Sinedino et al., 2014	CAN	194	211	180	225	PAG	28	TRUS	28
Piechotta et al., 2011	DEU	135	34	135	34	PSPB	26-58	TRUS	26-58
Romano et al., 2010	USA	113	133	114	132	PSPB	28	TRUS	28
Holtz et al., 2019	DEU	195	87	160	122	P4	21	RP	56
Lee et al., 1996	PRK	21	24	22	23	P4	21	RP	60
Wu et al., 2014	CHN	29	25	30	24	P4	19-23	RP	60
Faustini et al., 2007	ITA	679	312	565	426	P4	21	RP	42
Abdulla et al., 2014	IND	13	34	13	34	TRUS	30	TRUS	45
Chaudhary et al., 2012	IND	64	20	52	32	TRUS	25	TRUS	55
Nation et al., 2003	AUS	333	164	342	155	TRUS	28-35	TRUS	60
Quintela et al., 2012	SPN	691	60	684	67	TRUS	27-29	TRUS	56
Romano et al., 2006	USA	46	111	42	115	TRUS	28	TRUS	36
Scenzi et al., 1999	HUN	59	50	65	44	TRUS	29-30	TRUS	53-58
Scenzi et al., 1995	HUN	76	24	76	24	TRUS	27-29	TRUS	55-59
Scully et al., 2014	IRL	42	51	42	51	TRUS	21	TRUS	30

[1] ISG15= Interferon Stimulated Genes 15, PAG=Pregnancy Associated Glicoproteins with ELISA, PSPB= Pregnancy specific protein B, TRUS= Transrectal Ultrasound
[2] RP= Rectal Palpation

methodologies rather than the gold standard. However, these methodologies (ISG, PAG and TRUS) tell us that there is no significance ($p>0.05$) because the confidence interval coincides with the line of no effect (OR=1), in other words, there is no association. On the other hand, the P4 method has a significant result ($p<0.05$) in which the OR and the confidence interval is over 1. This means that the P4 is more accurate method to detect pregnancy than rectal palpation.

The ISG group consisted of 8 articles with 597 AI comparing ISG method at day 20 post insemination versus TRUS for pregnancy detection at day 30 of gestation. The OR>1 (OR= 1,1; 95%CI=0.78-1.56; P=0.52), favoring the ISG as a method for detecting pregnancy, but it is no significant. There was moderate heterogeneity (I^2=41.9%) between the 8 studies. Among the different methodologies, this subgroup has the lowest weighting (11.6%), meaning that even though it was evaluated 8 studies, the number of animals evaluated is small.

The PAG group consisted of 15 articles with 5781 AI detecting pregnancy at day 28. The gold standard used was TRUS at day 35 of gestation. The OR>1 (OR= 1,07; 95%CI=0.99-1.16; P=0.09), meaning that it is more accurate to detect pregnancy using PAG rather than TRUS. However, it is not significant. It can be observed that as a technique of pregnancy diagnosis, PAG is a method with several publications and with larger sample sizes (n=40-800). In this article, the weighting is 58.4%. The heterogeneity is no important (I^2=29.9%).

The evaluation of the P4 method include 1372 inseminated cows and diagnose pregnant at day 21 of gestation, the gold standard is rectal palpation at day 55. The OR>1 (OR= 1,58; 95%CI=1.18-2.11; P=0.02), meaning that a better method to detect pregnancy is P4 against RP. The weight of this subgroup is 11.8%. The heterogeneity is low (I^2=0.2%).

On the last scenario, we are comparing the same method, ultrasound, but at different time, 25 days post insemination versus 45 days in gestation. This subgroup included 1838 inseminated cows. The OR>1 (OR= 1,03; 95%CI=0.86-1.22; P=0.73). There is no significant result, however analyzing only the OR, there is a small advantage of detecting pregnancy by the TRUS at day 24 than at day 45 of gestation. The weighting of these subgroup is 18.21%. The heterogeneity is low (I^2=0%) between 8 studies.

4. DISCUSSION

The results from the present study indicate that all the methodologies evaluated are better diagnosing pregnant cows than the gold standard (RP and TRUS). Transrectal ultrasonography is regarded as the gold standard for determining earlier pregnancy status in cattle because it provides potential for visual and morphological assessment of the uterus, ovaries, embryo, and fetus (Speckhart et al., 2018). However, not all these results are significant. It is important to notice that the studies selected were chosen by high sensibility and specificity,67%-100% and 62%-100%, respectively. Meaning that there are reliable studies.

The OR for the ISG, PAG and TRUS exceed the value of 1 in small amounts. The only methodology that has a significant result is the methodology of P4. As the OR value exceeds 1 by a greater magnitude, it means that the evaluated method detects a greater number of pregnant cows compared to the standard gold. This numerical difference would mean that all animals initially diagnosed as positive (pregnant cows), suffered embryonic/fetal mortality, or were diagnosed as false positives.

Diskin & Morris (2008) proved that early embryo loss is greater in the high producing dairy cow rather than heifers and lower yielding dairy cows. Therefore, it is logical to

Table 2. Odds ratio, index of inconsistency and weighting of each method for early pregnancy diagnostic.

Type	ID	Reference	OR	Inferior	Superior	p value	Weighting %
ISG	1	Melo et al., 2020	0.64	0.40	1.02	0.06	2.52
	2	Han et al., 2006	1.55	0.81	2.96	0.19	1.53
	3	Yoshino et al., 2020	1.52	0.86	2.70	0.15	1.88
	4	Yoshino et al., 2018	0.93	0.45	1.94	0.85	1.25
	5	Wijma et al., 2016	2.03	1.00	4.12	0.05	1.33
	6	Serrano et al.,2020	1.09	0.48	2.45	0.84	1.06
	7	Pugliesi et al., 2014	0.82	0.44	1.52	0.53	1.67
	8	Soumaya et al.,2016	1.00	0.23	4.31	1.00	0.36
	I^2 :41,9%	Subgroup global	1.10	0.78	1.56	0.80	
PAG	9	Dufour et al., 2017	1.17	0.91	1.50	0.23	4.92
	10	Karen et al., 2015	0.88	0.50	1.56	0.66	1.90
	11	Kaya et al., 2016	0.92	0.42	2.03	0.84	1.11
	12	Mayo et al., 2016	1.06	0.78	1.45	0.69	4.08
	13	Mayo et al., 2016	1.03	0.75	1.40	0.87	4.08
	14	Meziane et al., 2021	1.11	0.45	2.71	0.82	0.89
	15	Moussafir et al., 2018	1.34	0.87	2.07	0.19	2.77
	16	Piechotta et al., 2011	0.93	0.55	1.57	0.79	2.16
	17	Shephard et al., 2018	1.22	1.01	1.47	0.04	5.90
	18	Silva et al., 2007	1.15	0.95	1.38	0.15	5.90
	19	Green et al., 2009	1.19	0.95	1.49	0.13	5.29
	20	Filho et al., 2020	0.74	0.60	0.92	0.01	5.48
	21	Commun et al., 2016	1.24	0.70	2.20	0.47	1.86
	22	Commun et al., 2016	1.13	0.64	2.01	0.66	1.88
	23	Sinedino et al., 2014	1.15	0.87	1.52	0.32	4.53
	24	Piechotta et al., 2011	1.00	0.59	1.70	1.00	2.09
	25	Romano et al., 2010	0.98	0.69	1.40	0.93	3.54
	I^2 :29,9%	Subgroup global	1.07	0.99	1.16	0.09	
P4	26	Holtz et al., 2019	1.71	1.21	2.41	0	3.64
	27	Lee et al., 1996	0.91	0.4	2.09	0.83	1.02
	28	Wu et al., 2014	0.93	0.43	1.98	0.85	1.19
	29	Faustini et al., 2007	1.64	1.37	1.97	0	5.97
	I^2 :0,2%	Subgroup global	1.58	1.18	2.11	0.02	
TRUS	26	Abdulla et al., 2014	1.00	0.41	2.47	1.00	0.87
	27	Chaudhary et al., 2012	1.97	1.01	3.84	0.05	1.47
	28	Nation et al., 2003	0.92	0.71	1.20	0.54	4.67
	29	Quintela et al., 2012	1.13	0.78	1.62	0.52	3.44
	30	Romano et al., 2006	1.13	0.69	1.86	0.62	2.33
	31	Scenzi et al., 1999	0.80	0.47	1.37	0.41	2.06
	32	Scenzi et al., 1995	1.00	0.52	1.91	1.00	1.53
	33	Scully et al., 2014	1.00	0.56	1.78	1.00	1.84
	I^2 :0%	Subgroup global	1.03	0.86	1.22	0.73	

think that the sooner pregnancy is detected, the greater the number of pregnant animals would be found, compared to diagnosing subsequent pregnancy to the same animals.
For example, for this study, the P4 detection method turned out to be significantly positive (OR>1), meaning that this method detects a higher number of pregnant cows at day 20, compared to rectal palpation at day 55 of gestation.

Considering the most representative studies, a higher percentage of false positive (FP) was observed in studies whose method of diagnosing pregnancy is progesterone measurement. Melo et al. (2020) reported a FP of 4% (6/144) when evaluating the ISG method at day 20 as a pregnancy diagnosis. Silva et al. (2007) and Shephard et al. (2018) determined low percentages of false positives 6% (49/879) and 5% (42/882), respectively using the PAG method at day 28. To detect pregnancy at day 25 by TRUS, Nation et al. (2003); reported FP of 1% (4/497). On the other hand, Faustini et al. (2007) and Holtz et al. (2019) found that the FP value doubles the previous methods, 14% (1/54) and 13% (124/991), respectively, when evaluating the detection of pregnancy by the P4 method.
Speckhart et al. (2018) establishes that detection of pregnancy by progesterone represents a risk for yielding false positive results in cows that have longer luteal phases, ovarian cyst, a prolonged corpus luteum, or embryonic mortality leading to challenges of nonreturn rates.
Otherwise, when the OR value is closer to 1 it means that the number of pregnant cows does not vary significantly in the two evaluations. That is, the number of pregnant diagnosed cows will be maintained until the second diagnostic method (gold standard). In this study, the TRUS method had the nearest OR to 1. A decrease of 0.6% was seen when comparing the number of pregnant cows at day 25 versus the number of pregnant cows at day 45 when analyzing the following studies: Scenzi et al., 1995; Scenzi et al., 1999; Nation et al., 2003; Romano et al., 2006; Chaudhary et al., 2012; Quintela et al., 2012; and Scully et al., 2014; Abdulla et al., 2014. On the other hand, Lee et al. (1996), Faustini et al. (2007), Wu et al. (2014) and Holtz et al. (2019) used P4 method for pregnancy detection. A decreased of 16% was seen when evaluating pregnant cows from day 21 to day 55, with an OR= 1.58, exceeding the unit with this method.

This difference in the decrease in pregnant animals between the two studies may be due to several factors like the time when pregnancy detection is taken. Late embryo loss is numerically lower than early embryo mortality, although, it causes serious economic losses in all production systems (Diskin et al., 2016). Reese et al., (2020) determined that by the conclusion of the first month of gestation, 47.9% of the cows submitted to a single insemination will not be pregnant. After the second month of gestation, the early fetal mortality will be 5.8%. Meaning that the embryo mortality is greater during the first month of gestation. In this scenario, we detected more embryo mortality during P4 method (21d- 55d post IA) versus TRUS method (25d-45d post IA). Another factor that also influences is the method of pregnancy detection. Progesterone is a steroid hormone that is produced by the corpus luteum to maintain pregnancy. It is considered as a marker that is not pregnancy specific because it is produced under other physiological conditions (Speckhart et al., 2018). Diagnosing pregnancy based on progesterone profiles is possible with high precision (sensitivity 95%) and determined early non pregnancy based on the spontaneous cessation of luteal phase between days 27 and 54 after AI. However, specificity values were less than 90% before day 40 (Bruinjé & Ambrose, 2019).

We need to consider that P4 it is not reliable to use as a single method of pregnancy diagnosing. To provide a more accurate diagnosis, this technology could be easily applied through the length of the entire embryonic period in dairy cattle. These requires labor- intensive manual sampling methods. The accuracy of prediction is substantially improved by analyzing additional samples. When taking progesterone concentrations on the 3rd week after insemination followed by additional determination 4 weeks after the initial sample (7th week in gestation) into consideration, the test accuracy was increased from 85% to 92% (Holtz & Niggemeyer, 2019).

5. CONCLUSION

- The TRUS method is the one that has the greatest similarity with the gold standard when it comes to pregnant cows, followed by PAG and ISG.
- The ISG, PAG, P4 and TRUS methodologies are better diagnosing pregnant cows than the gold standard (RP and TRUS). Nevertheless, the only method that is significant is the progesterone.
- Detecting pregnancy by the progesterone method may be affected by high rates of false positive or embryo mortality.

DECLARATIONS OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

NV, EM - The conception and design of the study, or acquisition of data, or analysis and interpretation of data,

NV, EM - Funding acquisition y project administration

NV, EM - Drafting the article or revising it critically for important content,

NV, EM - Final approval of the version to be submitted.

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