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Article

Effect of galectin-1 administration on pregnancy rate and its economic viability in TAI procedures in beef cattle

Efeito da administração de galectina-1 na taxa de prenhez e sua viabilidade econômica em procedimentos de TAI em bovinos de corte

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Abstract: The purpose of this work is to demonstrate the reproductive efficacy and the economic impact of a complementary tool to the TAI (timed artificial insemination) protocols - the administration of a single dose of recombinant human galectin-1 (rHGAL1) during the insemination procedure. GAL-1 can be considered as a modulator of the pregnancy development process. Reproductive efficacy was verified through the pregnancy rate in the first service, by ultrasonography (at 28-35 days) in contemporary groups (YG) of cows multiparous and with calf at the foot (from 60 to 100 days old) subdivided into 02 experimental groups (Control Group, composed of cows inseminated in a conventional, nCG = 1335 and Treated group, composed of cows inseminated with rhGAL-1 administration following the deposition of the semen dose, nTG = 1790). Ninety YG were formed, grouping cows under identical conditions (inseminator, farm/lot, breed, animal category, semen/bull variables). The experiment was conducted in 15 farms, with 3,125 cows (Nellore and crossbred), all and that maintained a body score (BSC) between 3.5 and 2.5 in the act of the TAI protocol and the pregnancy diagnosis. It was demonstrated, by the proposed statistical method (Generalized Linear Model (GLM) assuming a binomial distribution "pregnant" or "not pregnant") for residual effect under logarithmic function, PROBIT, which model included the "fixed effect" of YGs and treatments (dose 0 or dose 200), that the "rhGAL-1 dose" effect was significant, increasing the probability of obtaining pregnancy by 8.38 percentage points ($p < 0.0001$). Based on the construction of a hypothetical model, the economic profitability that can be obtained was compared. Using the average increase obtained by 8.38 percentage points more in the pregnancy rate, it was possible to increase productivity, adding, almost U$ 2 thousand to profitability for every 100 cows worked.

Keywords: economic profitability; fertility; proteins; reproductive biotechniques.

Resumo: O objetivo desse trabalho foi demonstrar a eficácia reprodutiva e o impacto econômico de uma ferramenta complementar aos protocolos de IATF - a administração de uma dose única de galectina-1 humana recombinante (rHGAL1) durante o procedimento de inseminação. A galectina-1 pode ser considerada como moduladora do processo de desenvolvimento gestacional. A eficácia reprodutiva foi verificada através da taxa de prenhez no primeiro serviço, por ultrassonografia (aos 28-35 dias) em grupos contemporâneos (YG) de vacas, multíparas e com bezerro ao pé (entre 60 e 100 dias de idade), subdivididas em 02 grupos experimentais (Grupo Controle = GC, composto por vacas inseminadas de modo convencional, nCG = 1335 e Grupo Tratado = GT, composto por vacas inseminadas com administração de rhGAL-1 na sequência da deposição da dose de sêmen, nTG = 1790). Noventa YG foram formados, agrupando-se vacas sob condições idênticas quanto às variáveis inseminador, fazenda/lote, raça, categoria animal, sêmen /touro. O experimento foi conduzido em 15 fazendas, com 3.125 vacas (Nelore e Cruzadas) que mantiveram score corporal (BSC) entre 3,5 e 2,5 no ato do protocolo de IATF e no diagnóstico de gestação. Foi demonstrando, pelo método estatístico proposto (modelo linear generalizado assumindo distribuição binomial "prenhe" ou "não prenhe" com efeito residual sob função logarítmica PROBIT, com modelo incluindo "efeito fixo" dos YG e tratamento (dose 0 ou 200) que o efeito "dose rhGAL-1" foi significativo, aumentando a probabilidade de obtenção de prenhez em 8,38 pontos percentuais ($p < 0,0001$). Baseado na construção de modelo hipotético, comparou-se a lucratividade e rentabilidade econômica que podem ser obtidas. Utilizando-se o incremento médio obtido de 8,38 pontos percentuais a mais na taxa de prenhez, pôde-se aumentar a produtividade, agregando-se, quase que U$2mil à lucratividade a cada 100 vacas trabalhadas.

Palavras-chave: biotécnicas da reprodução, fertilidade, proteínas, rentabilidade econômica.

Introduction

The economic impact of falls (reduction) in the pregnancy rate in herds, under the animal productivity scenario, is scientifically recognized (Pires et al., 2004) and the purpose of this work was to demonstrate an auxiliary tool to the TAI (timed artificial insemination) protocols, in increasing the pregnancy rate in beef cattle (administration of exogenous rhGAL-1 - recombinant human Galectin 1) and the economic impact obtained by increasing reproductive efficiency with the use of this tool.

A dose of 200 ± 10μg of rhGAL-1 diluted in 200μL of PBS 1X pH 7.0 buffer containing 50μg/ml kanamycin sulfate as a stabilizer was administered using the commercial product Tolerana Bovinos® (Inprenha Biotecnologia). The recommendation for administration of the product is the simple deposition of the dose in the lumen of the uterus, during the insemination after the application of the semen.

Galectin-1 is part of a family of proteins referred to as lectins (Barondes et al., 1994a, 1994b), which presents important physiological roles for the development and maintenance of pregnancy, acting as a modulator of the gestational development process (Blois et al., 2019), a fact that may corroborate the hypothesis of exogenous administration of rhGAL-1, as human recombinant protein (rhGAL-1) as recommended by the manufacturer of Tolerana Bovinos®.

There are several reports in the literature on the effect of GAL-1 on reproductive physiology, and its impact on pregnancy development, such as modulation of maternal-fetal immunotolerance (Hyde and Schust, 2016); vascular remodeling of the uterus and placental angiogenesis that aims at adequate oxygenation to the fetus (Cross et al., 2002); trophoblast invasion and placentation (Blois et al., 2019); in addition to participating in more generic effects such as adhesion, cell cycle proliferation, apoptosis, RNA metabolism and inflammatory process control (Perillo et al., 1995; Liu et al., 2002; Dias-Baruffi et al., 2003; Rubinstein et al., 2004; Stowell et al., 2007; Ramhorst et al., 2012; Barrientos et al., 2014; Blois et al., 2019). All these physiological processes can have consequences for increasing the embryonic mortality rate, which is considered as one of the main causes of the reduction in the pregnancy rate, as already stated by (Diskin and Morris, 2008; Diskin et al., 2016). Other causes commonly mentioned of embryonic mortality are diseases (Cheng et al., 2016), nutrition and milk production (Abdalla et al., 2017; Alfieri and Alfieri, 2017), lethal genetic mutations (Pohler et al., 2016), homeostasis placental and uterine environment (Farin et al., 2006; Pohler et al., 2016; Pohler et al., 2020), imbalance of immunological factors at the maternal-fetal interface (Than et al., 2008; Bidarimath and Tayade, 2017). Thus, the scientific basis corroborates the hypothesis of an increase in the pregnancy rate with the exogenous administration of rhGAL-1.

Material and Methods

Experiment Location

The experiments were conducted on 15 commercial beef cattle farms, located in different municipalities in Brazil (Campo Grande – MS; Naviraí – MS; Água Clara – MS; Formoso do Araguaia – TO; Gurupi – TO; Paragominas – PA; Uberaba – MG, Uberaba – MG; Pedregulho – SP; São Gotardo – MG; Prata – MG; Água Clara – MS and Cuiabá – MT). It should be noted that the farms chosen to carry out this work are farms that have performed artificial insemination procedures for at least two years.

Design of the experiment

The effectiveness of rhGAL-1 administration can be verified through the pregnancy rate in the first service (with one insemination), by ultrasonography (at 28-35 days) in contemporary groups (YG) of cows subdivided into 02 experimental groups (Control Group = GC and Treaty Group = TG). CG consisted of cows inseminated conventionally, with only a dose of semen, nCG = 1335. GT was composed of cows inseminated and administered rhGAL-1 following deposition of the semen dose, nTG = 1790. The product was administered only in first service protocols so that the cows might be on the same management level and so that the economic analysis calculation could be expedited. Due to the results of previous experiments (unpublished data), there is no difference for impact with the use of eGAL-1 administration either in the first, second, or third services. The purpose of this article is the discussion of economic impact based on the construction of hypothetical scenarios.

Galectin-1 (GAL-1)

Recombinant human galectin-1 (rhGAL-1) was manufactured by Inprenha Biotecnologia® (Tolerana Bovinos®). The definition of a rhGAL-1 dose (as an effective dose) was determined in previously conducted (unpublished) works, where different amounts of administered protein were tested, in addition to different times and forms of application. Once these parameters were defined, this experiment was designed. A dose of 200 ± 10 μg of rhGAL-1 is diluted in 200 μL of PBS 1X pH 7.0 buffer containing kanamycin sulfate at 50 μg/mL as a stabilizer. The recommendation for administration of the product is the simple deposition of the dose in the lumen of the uterus, during the insemination after the application of the semen 01 dose/cycle/cow was used (according to the package insert leaflet of the product Tolerana®).

Animals

The experiment was conducted in 15 different herds (farms) of beef cows managed under the extensive system, maintained in native and cultivated pasture and mineral supplementation. The cows were submitted to reproductive management for 3 months, including TAI protocols and natural breeding as a transfer. Prophylactic management with vaccination for HVB-1, BVD, and Leptospirosis (annual vaccination with dose plus “booster”) was optional management used by some of the farms that participated in the experiment. As it is exercised with the distribution of dams in treatment groups within YGs, the impact of management variables (vaccination, feeding, supplementation, among others) on the treatment effect (using or not using rhGAL-1) it is distributed equally among the YG.

Only cows that maintained a body score (BSC) between 3.5 and 2.5 were considered able to participate in the statistical analysis. The BSC classification criteria used were those described by Machado et al. (2008).

The experiment considered only multiparous cows with calves at foot, aged 60-100 days.

A total of 3,125 beef cows (Nellore breed or half Nellore crossbreed) were used in the experiment and divided into 90 contemporary groups (YG) and these were randomly divided into 02 treatment groups (TG = treated group, nTG = 1790 and CG = control group, nCG = 1335). The description of the construction of the YG is detailed as follows.

TAI and rhGAL-1 administration

The cows were kept in batches within each farm. Each batch was submitted to the TAI protocols that each farm chose. There were no impositions as to what hormones or doses should be used in the experiment. The prerogative was that the same protocol was used in both treatment groups, and them into the YGs. Details of the hormone protocol used by each farm are described in Table 1.

Another option for choosing each farm was to select the semen that each batch would use. The same concept was used to select the inseminators who would participate in the experiment. There was previous training with inseminators on how to apply the dose of rhGAL-1. In total 75 different bulls and 20 inseminators were considered in the experiment and made up the YGs.

There was also no prior selection of which cows that would make up the TG or CG, that means would or wouldn’t receive the dose of rhGAL-1. The option was random and followed the entry of the cows in the cattle crush – if the first cow received the dose of rHGAL1, the second that would enter would not receive such dose, so the first was part of the TG and the second of the CG and so on until the end of the batch. The administration of rhGAL-1 was just a simple deposition of the protein solution in the uterine lumen, performed after the deposition of semen and in exactly the same way as a second application of semen dose since the dose of the product is presented in straws of 0.25 mL, as well as semen.

Table 1. Farm (in letter codes), days of estrus synchronization protocols (D0= day zero, D7 = day seven, D8= da eight, D9 = day nine, TAI day = day of the AI procedure (CG) and eGAL-1 and respective hormones applied, with amounts administrated, according to the estrus synchronization protocol adopted by the partner farm

Farms Codes	Category	D0	D7	D8	D9	TAI day
K	M	EB 2.0mL	-	-	$PGF_{2\alpha}$ 2.0mL ECP 1.0mL eCG 1.5mL	D11
B	M	EB 2.0mL	-	$PGF_{2\alpha}$ 1.5mL ECP 0.5mL eCG 1.5mL	-	D10
C	M	EB 2.0mL	$PGF_{2\alpha}$ 2.0mL	-	ECP 0.5mL eCG 1.5mL	D11
G H I J L M N O P Q R S	M	EB 2.0mL	-	$PGF_{2\alpha}$ 2.0mL ECP 1.0mL eCG 1.5mL	-	D10

EB – Estradiol Benzoate. $PGF_{2\alpha}$ – Prostaglandin F2alpha. EC- Estradiol Cypionate. eCG – Equine Chorionic Gonadotropin.

Definition of pregnancy rate in experimental groups

The pregnancy rate was determined by ultrasonography diagnosis, performed from 28 to 35 days after TAI and the statistical difference between the experimental groups was observed considering the formed contemporary groups, as described below.

Contemporary Groups and Statistical Analysis

To define the difference between the pregnancy rate between the experimental groups (inseminated with and without the single dose of rhGAL-1), comparisons were made within contemporary groups (YG). The contemporary groups were composed of cows of the same breed and animal category, inseminated by the same technician (inseminator), belonging to the same management batch within the same farm, inseminated with the same bull (semen sample). The YGs were formed under minimum rules of composition and must be composed of a minimum number of 5 cows per group and in those where there was variation in the effect (pregnancy rate). In other words, contemporary groups with less than 5 cows and which presented a 100% or 0% pregnancy rate did not participate in the statistical analysis, a fact that explains the different number of cows between the CG and TG groups.

For the statistical analysis, the Generalized Linear Model (GLM) was applied, using the SAS GENMOD procedure (version 9.3), assuming a binomial distribution (pregnant or not pregnant) for residual effect under logarithmic function (PROBIT). The model included the "fixed effect" of YGs and treatments (dose 0 or dose 200). Dose 200 refers to the administration of rhGAL1 in TG cows, which is equivalent to the administration of a dose of rhGAL1 of 200 ± 10 µg, according to the definition in the authors' previous yet unpublished experiments.

In these conditions, 90 YG were formed with the arrangement of these 3125 cows, 75 bulls, and 20 inseminators in several management lots, into those 15 farms. From the PROC GENMOD statistical model, the probability of obtaining pregnancy under the dose effect (0 or 200 µg of rhGAL-1) administered in addition to the TAI protocol in the different YGs and the total group scenario was calculated.

Economic Viability Analysis

For the economic evaluation of TAI-based reproductive management, some performance indicators of the beef cattle described by Costa et al. (2018) and the use of this innovation, the administration of a dose of rhGAL-1 concomitantly with the execution of the first TAI service were considered. A hypothetical scenario was created, defining some reproductive productivity parameters and financial parameters to profitability to be calculated, namely:

a) 48% Pregnancy rate per service;

b) Cows being handled in a breeding season with up to 3 TAI services; comparative calculation was chosen using the prerogative of carrying out up to 3 services, within the breeding season, as it is the most used model, currently in Brazilian farms;

c) Discard cows that remained empty at the end of the breeding season;

d) Purchase of heifers to replace discarded cows;

e) Synchronized cow cost = considering the average Brazilian market value of a heat synchronization protocol plus one dose of commercial semen = U$ 10.36 (Santos et al., 2018, considering price rate in Mar/2/2021);

f) Number of empty cows at the end of the SB – which will be discarded (sold for slaughter) with a price calculated on it;

g) Number of TAIs performed in the SB. = calculation from the sum of the 3 TAI protocols performed during the entire Breeding-Season;

h) Number of calves obtained in SB. = calculation from the formula = (g) x (a%);

i) Cost/calf born = calculation from the formula = ((c) x (e)) / (h);

j) Expenses with calves born in the batch = (h) x (i);

k) Calf revenue = calculation from the formula = (j) x U$ 446.97 where this was the average Brazilian market value of a calf weaned at 8 months with 6@ (Scot Consultoria, 2021);

l) Breeding stock replacement cost = replacement heifer acquisition cost less revenue from selling empty cows at the end of the breeding season. Replacement female value, Nellore breed, at 18m with 8.5@ with a value of U$ 503. The commercial value of the cow for slaughter was considered the value of the cowherd, Nellore breed, with 10.5@ valued at U$ 533.00;

m) Profitability of the lot at the end of the SB. = calculation from the formula = (k) – (i) – (l), considering both groups (CG = just using semen dose and TG = using semen doses plus a Tolerana dose cost. Tolerana doses cost of U$ 3.00;

n) Exceeding profit obtained = (mGC) only cows on the CG, or - (mTG) only cows on TG.

The calculations of the economic analysis were carried out considering all the aforementioned parameters and with a simulation of an increase of 1 to 17 percentage points in the pregnancy rate, that is, simulating scenarios obtaining pregnancy rates from 49 to 65%.

For purposes of comparison of the impact of the use of rhGAL-1 on profitability, the same fixed maintenance costs of the breeders per farm were considered for both experimental groups (TG and CG), and for this reason, they are not highlighted in the calculations. The fixed maintenance costs is so particular for each farm that is not the purpose of this analysis.

The economic evaluation was carried out considering the US dollar currency, using the official conversion rate, between Real and US dollar, of March 2021. This decision was based on avoiding errors in future interpretations, as a result of the possibility of exchange volatility of the real against the dollar, considered the strongest currency internationally.

Ethics Committee

The present study followed the ethical criteria for the use of animals in scientific experiments and was approved by CEUA/USP protocol number 11.1.95.53.5.

Results

Pregnancy Rates Obtained

To find the value of the increment in percentage points on the pregnancy rate in TAI procedures with the use of rhGAL-1, the experiment was conducted in different environments, as described in "material and methods", however, with all variables considered in the statistical model, since the distribution of treated (with the dose of rhGAL-1) and untreated cows within contemporary groups was established. The discussion on the effect of the usage of rhGAL-1 on the pregnancy rate in inseminated beef cows will not consider the simple comparison of the average pregnancy rate of each experimental group. Despite this, just as descriptive, Table 2 shows the average pregnancy rate obtained in the CG (42.72%) and the TG (57.28%). We believe that there are important variables and to be considered when comparing the 2 experimental groups. For this reason, the comparative weighting within the contemporary groups formed.

Table 2. Pregnancy rate (%P) by ultrasonography at 28-35 days after timed artificial insemination (TAI) of the cows submitted (TG) or no (CG) at administration of recombinant human galectin 1 (rhGAL-1) during the insemination, and probability (%PRO) of obtaining pregnancy under the "rhGAL-1 usage effect", and (SE) is the standard error of the probability of average pregnancy rate.

Groups					
	CG			TG	
NCG	%PGC	%PROCG (SE)	NTG	%PTG	%PROTG (SE)
1335	42.72	50.65^{a} (0.01889)	1790	57.28	59.03^{b} (0.01777)

a, b indicated that probability of obtaining pregnancy under the effect of "fhGAL-1 usage" differs statistically (p< 0.0001) into those groups (CG and TG), based on the methodology used

Source: Elaborated by the author

The results obtained by the statistical analysis based on the construction of contemporary groups (YG), aiming to fix all the variables that could interfere in the pregnancy rate, in addition to the "effect of using or not using rhGAL-1 administration. It was demonstrated that the "rhGAL-1 usage" effect was statistically significant over the "pregnancy" effect ($p < 0.0001$), confirming that the administration of the single dose of rHGAL1 brought a positive effect on the increase in the pregnancy rate (biological effect confirmed); while the analysis demonstrated that the YG effect was no difference statistically ($p < 0.241$). This is positive as the 90 YG may not have affected the variable response and it will be discussed after.

However, the statistical method used calculated that the probability of obtaining pregnancy under the "rhGAL-1 usage effect" (which means the administration of rHGAL1), rose 8.38 percentage points in the TG. The result was obtained by comparing the probability of obtaining pregnancy in the TAI+rhGAL-1 protocol (59.03%) and the probability of obtaining pregnancy using a conventional protocol, (50.65%), based on the statistical model used (evaluating the desired effect within the created contemporary groups), as described at Table 2.

Economic Viability Analysis

All calculations performed, according to the parameters described in the "material and methods", are summarized in Table 3. A batch of 100 cows managed in the breeding season with up to 3 attempts to obtain pregnancy was assumed, hypothetically. For each new attempt, one additional TAI service was performed and computed in the cost. The cost of the synchronization protocol is the same, regardless of the number of services performed (first, second or third TAI in each cow).

The starting point of 48% was assumed as the pregnancy rate, number close to 50% that normally is considered in Brazilian beef cattle TAI market, and in Table 3, the results of hypothetical calculations were summarized, considering each added percentage point in the pregnancy rate, using the dose of rhGAL-1, and the aggregate cost with its acquisition, on the economic parameters of the breeder herd, considering mainly the (n) Exceeding profit obtained using rhGAL-1 against the group that does not use; and (m) profitability of the lot at the end of the SB using or not using rhGAL-1.

Table 3. Hypothetical comparison between batch of 100 cows being worked in a breeding station with up to 3 inseminations to obtain pregnancy, considering average pregnancy rate in the batch, using conventional TAI protocol (CG) and using TAI plus rHGAL1 (TG), demonstrating the number of TAIs performed in each event (1st, 2nd and 3rd IATF) and number of calves obtained at the end of SB, showing costs, revenues and more profit obtained with different scenarios of improvement in the pregnancy rate with the use of rHGAL1, at the end of SB. Where: %XP = % pregnancy average batch; (a) = synchronized cow cost, in US$; n cows for 1st, 2nd and 3rd TAI means cows for first, second or third service; (f) = number of empty cows at the end of SB; (g) number of TAIs performed during SB; (h) = number of calves obtained in SB; (i) = cost/calf born in US$; (j) = expenses with calves born in the batch; (k) = Revenue on calves; (l) = breeding stock replacement cost, in US$; (m) = profitability of the lot at the end of SB, in US$, (n) - Exceeding profit earned, in US$.

Protocol	rHGAL-1 US$/ dose	(a)	n cows for 1st TAI	% XP	n cows for 2nd TAI	n cows for 3rd TAI	(f)	(g)	(h)	(i)	(j)	(k)	(l)	(m)	Exceeding profit earned
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TAI (CG)	$0.00	$10.36	100	**48**	52	27	14	179	86	21.58	1,854.85	38,412.24	-421.82	36,979.21	
				49	51	26	13	177	87	27.27	2,364.85	38,767.90	-397.95	36,801.00	-178.22
				50	50	25	13	175	88	26.72	2,338.00	39,109.88	-375.00	37,146.88	167.66
				51	49	24	12	173	88	2620	2,311.41	39,438.44	-352.95	37,479.98	500.76
				52	48	23	11	171	89	25.69	2,285.09	39,753.87	-331.78	37,800.55	821.34
				53	47	22	10	169	90	25.21	2,259.04	40,056.42	-311.47	38,108.85	1,129.64
				54	46	21	10	167	90	24.74	2,233.26	40,346.37	-292.01	38,405.12	1,425.91
				55	45	20	9	165	91	2429	2,207.74	40,623.99	-273.38	38,689.62	1,710.41
				56	44	19	9	163	91	23.86	2,182.49	40,889.53	-255.55	38,962.59	1,983.38
TAI + rHGAL1 (TG)	$3.00	U$13.36	100	**57**	43	18	8	161	92	23.44	2,157.51	41,143.28	-238.52	39,224.29	2,245.08
				58	42	18	7	160	93	2303	2,132.79	41,385.49	-222.26	39,474.96	2,495.75
				59	41	17	7	158	93	22.64	2,108.34	41,616.44	-206.76	39,714.86	2,735.65
				60	40	16	6	156	94	22.27	2,084.16	41,836.39	-192.00	39,944.23	2,965.02
				61	39	15	6	154	94	21.90	2,060.25	42,045.62	-177.96	40,163.33	3,184.12
				62	38	14	5	152	95	21.55	2,036.60	42,244.39	-164.62	40,372.40	3,393.19
				63	37	14	5	151	95	21.21	2,013.22	42,432.96	-151.96	40,571.70	3,592.49
				64	36	13	5	149	95	20.88	1,990.11	42,611.62	-139.97	40,761.48	3,782.27
				65	35	12	4	147	96	20.55	1,967.26	42,780.62	-128.63	40,941.98	3,962.77

Source: Elaborated by the author

To calculate the values of (n) and (m) it was necessary to perform some previous calculations for productive efficiency parameters, such as: (g) number of TAIs performed in the SB, (h) number of calves obtained in SB, (i) cost/calf born and (j) expenses with calves born in the batch. However, some other parameters had values defined by the current Brazilian market, according to the consulted source, for example, parameters such as (a) synchronized cow cost (k) calf revenue, (l) breeding stock replacement cost. All values included on the calculation are from recognized sources at Brazilian market.

Table 3 shows the results obtained from the hypothetical calculations, considering 100 cows, as it facilitates the prospecting of calculations for larger herds, simply multiplying by the correction factor depending on the size of the batch of interest.

Discussion

Effect of the rhGAL-1 usage on the pregnancy rate

It is noted that a single dose of rhGAL-1 (200 ± 10μg), administered as recommended by the manufacturer, complementing an artificial insemination protocol, was effective in the probability of increase the pregnancy rate in beef cows by 8.38 percentage points and with considerable statistical relevance ($p < 0.0001$), in increasing the probability of "obtaining pregnancy" according to the statistical model constructed using YG to compare the "rhGAL-1 usage" effect. It is important to remember that the dose of rhGAL-1 used was previously determined in the authors' previous yet unpublished experiments. In these previous experiments, doses of 50, 100, 200, and 400 µg of rhGAL-1 were tested, administered at different times (with the TAI procedure, 7 or 12 days later) and with different procedures (types of applicators) and only after the definition of the effective dose and the best administration procedure is that this experiment and the statistical and economic comparisons were carried out.

It is worth noting that the main variables that can interfere in the pregnancy rate in IAT protocols were "controlled" in the proposed statistical model since the pregnancy observations were carried out among experimental groups (TG vs CG) divided between YG, reinforcing the hypothesis that the only variable responsible for the increase in the pregnancy rate was the use of rhGAL-1 during the TAI procedure, noting that the YG effect did not obtain statistical significance ($p = 0.24$) considering the data observed in the 90YG formed in this experiment.

Elucidating the reasons why rhGAL-1 can impact pregnancy rates, it is worth mentioning that:

a) From the mid-1970s onwards, several findings of animal lectins and β-galactoside ligands have been described. Barondes et al. (1994a) proposed the creation of the galectin family to group these proteins;

b) Galectins are a family of evolutionarily conserved proteins distributed from lower invertebrates to mammals (Cummings and Liu, 2009; Modenutti et al., 2019);

c) Than et al. (2008) showed that LGALS1 has a high degree of structural conservation, dimerization, and binding properties with carbohydrates and integrins (adhesion proteins), suggesting that these properties are conserved among vertebrates and that they maintain a pattern of gene expression among the different types of the placenta (deciduous or not). Thus, the efficacy of recombinant human Gal-1 under assisted reproduction procedures was evaluated, besides bovine females (this work), ovine and equine (unpublished data);

d) Literature data suggest that GAL-1 can be secreted by direct translocation of cytosol through the plasma membrane with the aid of cytosol and membrane factors (Schäfer et al., 2004; Nickel, 2005);

e) galectins are multifunctional molecules that participate in several biological processes such as adhesion, proliferation, and cell cycle, apoptosis, RNA processing, control of the inflammatory process, and physiological mechanisms of reproduction (Perillo et al., 1995; Liu et al., 2002; Dias-Baruffi et al., 2003; Rubinstein et al., 2004b; Stowell et al., 2007; Ramhorst et al., 2012; Barrientos et al., 2014; Blois et al., 2019);

f) The galectin's maternal-fetal tolerance role, both innate and adaptive, is associated with regulating and modulating the embryo elongation events' immunological responses and adherence to the endometrium (Farmer et al., 2008);

g) They also contribute to placentation as they regulate the development, migration, and trophoblastic invasion, essential in early gestational development (Barrientos et al., 2014; Blois et al., 2019, 2007; Freitag et al., 2013);

h) The interaction of GAL-1 with integrins suggests participation in the extracellular matrix and placentation events, either in the oxygen exchange and/or nutrients or by the vessels formation (angiogenesis), showing that GAL-1 plays a vital role in interface signaling maternal-fetal since it has multiple biological functions (Choe et al., 1997);

i) Blois et al. (2007) demonstrated high pregnancy loss rates in mice in which the Lgals1 gene was deficient (knockout mice). When treating deficient mice with recombinant GAL-1, there was a decrease in fetal loss and the restoration of tolerance through several mechanisms, including the induction of tolerogenic dendritic cells, which in turn promoted the expansion of regulatory T cells secreting interleukin-10 (IL-10) in vivo. Consequently, the protective effects of GAL-1 have been revoked in mice depleted of regulatory or IL-10 deficient T cells. Thus, Blois et al. 2007 demonstrated the fundamental importance of GAL-1 in fetomaternal tolerance and the synergy between GAL-1 and progesterone in maintaining pregnancy.

With this experiment, it was evident, in practical terms, that yes, that GAL-1, administered in the form of rhGAL-1 during the TAI procedure, significantly impacted the increase in the pregnancy rate in cows, corroborating all the technical arguments evidenced in the references cited above.

The economic impact of the pregnancy rate and pregnancy loss

The concept that reproduction plays a fundamental role in the efficiency and profitability of the beef cattle production system is already widely debated, Reese et al. (2020) describes a detailed meta-analysis on this subject. It is cited that the low production rates associated with temporary postpartum infertility (anestrus, which increases the birth-heat interval, birth-conception, and interval between births) are considered to be the main cause (Pires et al., 2004; Yavas and Walton, 2000).

It is also known that the use of biotechnologies can improve reproductive and consequently productive indexes, such as, for example, fixed time artificial insemination (TAI), whose main purposes are to increase the fertility of induced estrus, the ovulation precision (McKinniss et al., 2011; Tortorella et al., 2013), but which also adds other values (genetic and economic) with the use of genetically superior bulls, the concentration of farm labor, synchronizes and induces cyclicality of the animals, which increases the proportion of pregnancy at the beginning of the breeding season and consequently increases the recovery time for a new pregnancy in the following season (Sá Filho et al., 2013).

In addition, according to Bó et al. (2005) calves born from TAI at the beginning of the calving season are heavier at weaning, increasing the profitability of the producer. Therefore, due to the importance of the AI and TAI Technique for livestock, the present work aims to present how important is the increase in the pregnancy rate and its impact on the economic results of a managed batch of cows. Technicians who work daily with AI know very well how difficult it is to increase the pregnancy rate by 1 percentage point at the end of the breeding season, and even more, increase the pregnancy rate by 1 percentage point per service performed. Hence the technological relevance of innovations that may increase the pregnancy rate, as the administration of rhGAL-1 demonstrated, increasing the probability of obtaining pregnancy by another 8 percentage points, being evaluated in different scenarios (the 90 YGs analyzed).

Regardless of which breeding management is adopted on the property, costs will always exist (hormones and labor at the TAI, acquisition, and management of bulls in the natural range), so the decision of which model to choose must be based on technical and economic analysis, aiming at the increased production performance (Amaral et al., 2003). The present work aims to analyze whether the use of a complement to the TAI protocol, with the administration of rhGAL-1, impacts this increase in productive performance, comparing the pregnancy rates at each cycle of TAI and the cumulative pregnancy rate to the end of the breeding season.

Several studies compare the performance obtained under the pregnancy rate in different situations of reproductive management; Silva et al. (2007) evaluated the cost-benefit ratio of conventional AI versus TAI; Sá Filho et al. (2013) comparing pregnancy rate at the end of season breed. obtained in the management of TAI versus conventional AI and natural breed (NB), concluding that one of the great advantages of TAI with NB was the anticipation of pregnancy, increasing the recovery time of the female after childbirth for the next season breed., which increased the likelihood of new pregnancy and decreased the rate of disposal; Santos et al. (2018) who compared the economic profitability of NB versus TAI, and Paula et al. (2018) mention the improvement of reproductive biotechniques as a tool for improving the productive system as a whole, in addition to many other citations.

Management of TAI induces ovulation of cows in anestrus and can be considered the main cause for the highest pregnancy rate at the beginning of season breed., impacting on greater cyclicality due to an endocrine recovery of the female, which reflects a reduction in the possibilities of NB at the end of the breeding station (Santos et al., 2018). Furthermore, the anticipation of pregnancy for the start of the breeding season, allows the formation of lots of calves more homogeneous at weaning, with a higher morphological pattern and greater weight, points that increase the profitability of the producer who works with the calf. Bó et al. (2005) observed calves born from TAI protocols, 10 to 20 kg heavier than those born from MN.

According to Neto and Dalchiavon (2017), the use of TAI in beef cattle is a project of proven economic feasibility, as long as the total profitability values at the end of the project (sale and replacement of breeding stock) are considered. Based on this concept and adding the cost of the born calf, revenue from the sale of calves, the pregnancy rate of the batch in the first, second, and third inseminations, the economic comparison data with and without the use of rhGAL-1 doses will be presented as complementary to TAI protocols in beef cattle.

The method of calculating the cost of a pregnancy can vary considerably for situations of handling TAI, impacted by the strategic vision and/or degree of technification used by each technician and or property in question. However, the points considered as the "key points" in the calculation, are cost with hormonal protocol, cost of semen dose, the labor cost of handling TAI, and the pregnancy rate obtained at the end of the breeding season.

Profitability analysis using rhGAL-1 management

Next, a profitability analysis with the previously presented results and that can be obtained using a single dose of rhGAL-1 will be discussed, considering the calculations performed and results already presented in Table 3. As the premise was to compare the profitability between batches managed with and without the administration of rhGAL-1, animal maintenance values were not considered in the cost composition because the variable is the same between batches, regardless of whether or not they would receive the innovative product in handling. As each rural property has a very particular maintenance cost, we saw no reason to assume a value that does not translate as the reality of many. For those interested in closing the full cost, it is suggested to use the tables, considering their own maintenance cost.

It is notable among the calculations performed, and shown in Table 3, that from the increase of 2 percentage points in the pregnancy rate, using the administration of rhGAL-1, there is already Exceeding profit earned, albeit with an increase in the investment cost in TAI protocols. Recalling that 8.9 points were the average obtained in statistical analysis previously presented, this meant that the breeding stock replacement cost (l) was reduced from $421.82 to $255.55 and the profitability of the batch at the end of SB (m) increased from $36,979.21 to $38,962.59, a difference of $1,983.38 for each batch of 100 cows worked. If the managed herd is larger, just correct the calculation considering this fact, using a correction factor for the batch size.

In the same Table 3, it is possible to verify the cost of the calf born and the most profit obtained with a batch of 100 cows worked, comparing whether or not to use an administration of rhGAL-1, considering the different hypothetical scenarios with an increase from 1 to 17 percentage points in the pregnancy rate. Although the cost of the calf born can be higher (considering the hypothesis to improve the pregnancy rate until 14 percentage points), due to the purchase of the product, which adds value to the cost of the synchronized cow, the profitability of the batch is notable when analyzing the values of the most profit obtained, being able to profit up to more than U$ 3,9602.77 in the batch of 100 cows when 17 percentage points are added in the accumulated pregnancy rate of the batch (from 48 to 65%), recalling that the investment with the purchase of the product has already been counted in the cost of the synchronized cow. It is important to emphasize that the impact of using rhGAL-1 varies between the different scenarios in which the product was tested, within the 90YGs worked. The statistical model calculated the mean value, however, there was variation between the aggregated percentage points in the different YGs and therefore the hypothetical calculations were made with this variation margin.

As in any economic and financial estimate, the premises may change, depending on each rural property, the pregnancy rate at the first service, the pregnancy rate at the end of the SB., at the cost of the calf born at the end of the SB and of course here, at the potential for improvement in pregnancy rate using the complement proposed herein (rhGAL-1).

For Santos et al. (2018) for example, the cost of pregnancy can vary between U$ 30 to U$ 50 – whose calculation assumptions were based on the pregnancy rate (78 and 93% respectively) and the value of the semen dose used (U$ 3 to U$ 5 respectively). For Paula et al. (2018), the cost of pregnancy per calf ranged from US$ 23.17 to US$ 29.68 in TAI programs, corroborating the values suggested in the simulation above. Such authors demonstrated in their work a very detailed economic analysis on the use of TAI biotechnology, which is quite valid for our discussion herein.

It should be considered that an increase in the pregnancy rate is consistent with a reduction in pregnancy losses, which can occur in any gestational phase (the initial, middle and final third of pregnancy). However, it is a condition most commonly seen in the phase of the initial third of pregnancy, as described by Wiltbank et al. (2016). These authors describe, in a very detailed manner, the 4 phases/causes of pregnancy loss, which occur in the first gestational trimester in cows - the phase 1, which occurs in the first week after AI whose main causes are failures in fertilization/conception, low embryonic quality and/or early embryonic death, all as a consequence of environmental and hormonal issues; the phase 2, which takes place between the 8-27th day, includes the embryonic elongation phase and the classic period of "maternal recognition of pregnancy" whose main causes mentioned for such failures are the inefficient embryonic signaling, through interferon-tau and consequent non-maintenance of the CL, failures or delays in the elongation of the trophoblast resulting in a weak formation of the histotroph; the phase 3, from 28-60 days, whose causes mentioned are failures or delays in the development of chorion and alantodian placentonium; regression of CL or embryonic death; and the last phase 4, from 60 to 90 days, whose causes are related to gestational twinning in the same uterine horn. The same authors point out that in statistical terms phase 2 is the one with the highest occurrence of cases in the studied herds, adding up to about 25 to 41% of gestational losses, followed by phase 3 that represents about 12% of gestational losses.

In addition to the profitability obtained, previously mentioned, other economic aspects important to the increase in the productivity of a herd, in addition to the increase in the pregnancy rate at the end of Breeding Season using TAI + rhGAL-1, such as (i) reducing the interval between the birth of the herd; (ii) anticipating the conception of the batches and gradual correction of the Breeding Season; (iii) concentration of births and weaning at the best times of the year; (iv) increased weight at weaning; (v) reducing the age at slaughter; (vi) improvement in the standardization of the herd and carcasses; (vii) improvement in the control and direction of the Herd. The sum of these advantages will translate into greater profitability than previously calculated.

We emphasize that the results presented herein show a current reality, consistent with a history of experimentation, including stages of the development of any innovative product, with setbacks and challenges (dose adjustment, presentation, location/form/moment of application, active molecule stability, compatibility with heat synchronization protocols, among others) that did not always translate into effective results. However, nowadays, after this stage of experimentation, it is clear that the use of this technology (rhGAL-1 dose administration) can bring an enormous advantage to the profitability of a batch of cows in production. Therefore, the need for correct use is emphasized, following all the manufacturer's recommendations and that the product is indicated as a tool to increase productivity and not as a treatment for infertile and/or sick animals (including all those diseases that jeopardize fertility). It is believed that technology is a complementary tool and not the only "solution" for improving the pregnancy rate. Many variables must be considered (animal category, inseminator, semen fertility used, synchronization protocol, management, climate, nutrition, and herd health), all commented by Lamb et al. (2010) and Marques et al. (2018). It can be corroborated with such evidence, since the statistical model used demonstrated that contemporary groups significantly interfere in the question of pregnancy ($p < 0.001$), demonstrating that a lot can interfere with pregnancy results and, consequently, in the reproductive/productive efficacy of a herd.

It should be noted that there were no reports of adverse biological effects – discomfort, pain, irritability during and/or after administration of the product – or reports of problems with neonatal and stillborn malformations resulting from the use of the product.

Conclusions

The work herein showed that the administration of rhGAL-1 as a complement to TAI protocols in beef cattle, if used correctly, is effective in its purpose of use, improving reproductive efficiency of herds, since it increased the probability of obtaining pregnancy up to 8.38 percentage points. Consequently, it improves the increase in the productivity of beef cattle, adding approximately US$ 2 thousand more in profitability for a batch of 100 cows worked. It should be noted that the economic comparison between the treatment groups considered the same value of semen doses for both groups.

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