UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE AGRONOMIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOTECNIA

GIOVANI LUIS FELTES

DIFERENTES ABORDAGENS PARA A AVALIAÇÃO GENÉTICA DA PRODUÇÃO DE OÓCITOS E EMBRIÕES NA RAÇA GIR LEITEIRO

Porto Alegre
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“O conhecimento serve para encantar as pessoas, não para humilhá-las.”
Mário Sergio Cortella

DIFERENTES ABORDAGENS PARA A AVALIAÇÃO GENÉTICA DA PRODUÇÃO DE OÓCITOS E EMBRIÕES NA RAÇA GIR LEITEIRO[1]

Autor: Giovani Luis Feltes
Orientador: Jaime Araújo Cobuci

RESUMO:

As tecnologias de reprodução artificial, como a produção de oócitos e embriões, desempenham um papel importante na disseminação de material genético superior, contribuindo de forma efetiva para acelerar o ganho genético de características de interesse comercial no sistema de produção e no desenvolvimento da cadeia produtiva de carne e leite. Frente a isso, o objetivo deste estudo foi avaliar diferentes metodologias na avaliação genética da produção de embriões e oócitos em bovinos Gir Leiteiro e identificar as estratégias mais adequadas para futuras avaliações genéticas da raça. No primeiro capítulo, apresentamos a revisão de literatura sobre os temas que estão diretamente relacionamentos aos objetivos do nosso projeto. No segundo capítulo, utilizamos modelos de repetibilidade e regressão aleatória (RRM) para estimar parâmetros genéticos e predizer valores genéticos para a produção de oócitos e embriões, e identificamos os modelos que resultaram em maiores ganhos genéticos na produção de oócitos e embriões. No terceiro capítulo, investigamos o impacto do estresse térmico nos parâmetros genéticos e valores genéticos para produção de oócitos e embriões em animais da raça Gir Leiteiro. E, no quarto capítulo, avaliamos a eficiência dos modelos com distribuição Gaussiana (variável não transformada — LIN; transformada por logaritmo — LOG; transformada pelas distribuições de Anscombe — ANS) e Poisson (POI) na avaliação genética para a produção de oócitos e embriões. A herdabilidade estimada pelo RRM foi superior à estimada pelo modelo de repetibilidade, e o ganho genético foi maior, com acréscimos variando entre 0,02 (13,33%) e 0,26 (152,94%) ao utilizar o RRM. Observamos uma reordenação na classificação dos valores genéticos preditos dos 5% melhores touros e fêmeas ao longo da idade da doadora no momento da coleta de óocitos. É recomendável considerar o efeito do estresse térmico por meio dos índices de temperatura e umidade nas avaliações genéticas da raça para produção de oócitos e embriões e, assim, é possível selecionar animais tolerantes ao estresse térmico em características relacionadas à reprodução artificial. Também observamos o reordenamento dos melhores animais em relação às escalas de idades para coleta de oócitos e do índice de temperatura e umidade. Dessa forma, esses fatores devem ser levados em consideração no momento de identificar e selecionar os candidatos à reprodução. Ainda, identificamos que o modelo POI ajustou-se melhor aos dados e resultou em maior acurácia dos valores genéticos quando comparado aos demais modelos com distribuição de probabilidade Gaussiana (LIN, LOG e ANS). Os resultados obtidos nesta tese suportam a conclusão de que é possível obter progresso genético para a produção de oócitos e embriões por meio de seleção. Isso pode

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[1] Tese de Doutorado em Zootecnia, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil. (149 p.) Abril, 2022.

contribuir para o crescimento econômico de fazendas e demais empresas envolvidas na comercialização de embriões. Além disso, a rápida disseminação de material genético superior, proporcionada pelas técnicas reprodutivas via grande número de progênies de fêmeas com alto valor genético para diferentes características de interesse econômico, favorecem também o desenvolvimento e o retorno financeiro para os sistemas produtivos.

Palavras-chave: distribuição de Poisson, estresse térmico, índice de temperatura e umidade, modelo de regressão aleatória, modelo de repetibilidade, modelos lineares generalizados.

DIFFERENT APPROACHES FOR THE GENETIC EVALUATION OF OOCYTE AND EMBRYO PRODUCTION IN THE DAIRY GIR BREED[2]

Author: Giovani Luis Feltes
Adviser: Jaime Araújo Cobuci

ABSTRACT:

Artificial reproduction technologies, such as the production of oocytes and embryos, play an important role in the dissemination of superior genetic material, effectively contributing to accelerate the genetic gain of traits of commercial interest in the production system and in the development of the production chain of meat and milk. In view of this, the objective of this study was to evaluate different methodologies in the genetic evaluation of the production of oocytes and embryos in Dairy Gir cattle and to identify the most appropriate strategies for future genetic evaluations of the breed. In the first chapter, we present a literature review on topics that are directly related to the objectives of our project. In the second chapter, we used repeatability and random regression (RRM) models to estimate genetic parameters and predict breeding values for oocyte and embryo production, and we identified the models that resulted in the greatest genetic gains in oocyte and embryo production. In the third chapter, we investigated the impact of heat stress on genetic parameters and breeding values for the production of oocytes and embryos in Dairy Gir animals. And, in the fourth chapter, we evaluated the efficiency of models with Gaussian distribution (non-transformed variable — LIN; transformed by logarithm — LOG; transformed by the Anscombe distributions — ANS) and Poisson (POI) in the genetic evaluation for the production of oocytes and embryos. The heritability estimated by the RRM was higher than that estimated by the repeatability model, and the genetic gain was greater, with increases ranging between 0.02 (13.33%) and 0.26 (152.94%) when using the RRM. We observed a reordering in the classification of the predicted breeding values of the top 5% sires and females along the age of the donor at ovum pick-up. It is recommended to consider the effect of heat stress through the temperature-humidity index in the genetic evaluations of the breed for the production of oocytes and embryos and, thus, it is possible to select animals tolerant to heat stress in traits related to artificial reproduction. We also observed the reordering of the best animals in relation to the age scales for age at ovum pick-up and the temperature-humidity index. Thus, these factors must be taken into account when identifying and selecting breeding candidates. Furthermore, we identified that the POI model fitted the data better and resulted in greater accuracy of the breeding values when compared to the other models with Gaussian probability distribution (LIN, LOG and ANS). The results obtained in this thesis support the conclusion that it is possible to obtain genetic progress for the production of oocytes and embryos through selection. This can contribute to the economic growth of farms and other companies involved in the commercialization of embryos. In addition, the rapid dissemination of superior genetic material, provided by

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[2] Doctoral thesis in Animal Science, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. (149 p.) April, 2022.

reproductive techniques via a large number of progenies of females with high genetic value for different traits of economic interest, also favors the development and financial return for production systems.

Keywords: Poisson distribution, heat stress, temperature-humidity index, random regression model, repeatability model, generalized linear models.

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LISTA DE ABREVIATURAS E SIGLAS

A	*Additive genetic effect*
AIC	*Akaike Information Criterion*
ANS	*Anscombe model (transformed by Anscombe)*
AOPU	*Age at ovum pick-up*
BIC	*Bayesian information criterion*
CE	*Cleaved embryos*
CG	*Contemporary group*
DIC	*Deviance information criterion*
DBT	*Dry bulb temperature*
D7M2	*Random regression model that considers two regressors for genetic additive and permanent environmental effect at AOPU and THI, considering THI about 7 days.*
D7M3	*Random regression model that considers three regressors for genetic additive and permanent environmental effect at AOPU and THI, considering THI about 7 days.*
D7M4	*Random regression model that considers four regressors for genetic additive and permanent environmental effect at AOPU and THI, considering THI about 7 days.*
D14M2	*Random regression model that considers two regressors for genetic additive and permanent environmental effect at AOPU and THI, considering THI about 14 days.*
D14M3	*Random regression model that considers three regressors for genetic additive and permanent environmental effect at AOPU and THI, considering THI about 14 days.*
D14M4	*Random regression model that considers four regressors for genetic additive and permanent environmental effect at AOPU and THI, considering THI about 14 days.*
D28M2	*Random regression model that considers two regressors for genetic additive and permanent environmental effect at AOPU and THI, considering THI about 28 days.*

D28M3	*Random regression model that considers three regressors for genetic additive and permanent environmental effect at AOPU and THI, considering THI about 28 days.*
D28M4	*Random regression model that considers four regressors for genetic additive and permanent environmental effect at AOPU and THI, considering THI about 28 days.*
D42M2	*Random regression model that considers two regressors for genetic additive and permanent environmental effect at AOPU and THI, considering THI about 42 days.*
D42M3	*Random regression model that considers three regressors for genetic additive and permanent environmental effect at AOPU and THI, considering THI about 42 days.*
D42M4	*Random regression model that considers four regressors for genetic additive and permanent environmental effect at AOPU and THI, considering THI about 42 days.*
D56M2	*Random regression model that considers two regressors for genetic additive and permanent environmental effect at AOPU and THI, considering THI about 56 days.*
D56M3	*Random regression model that considers three regressors for genetic additive and permanent environmental effect at AOPU and THI, considering THI about 56 days.*
D56M4	*Random regression model that considers four regressors for genetic additive and permanent environmental effect at AOPU and THI, considering THI about 56 days.*
D112M2	*Random regression model that considers two regressors for genetic additive and permanent environmental effect at AOPU and THI, considering THI about 112 days.*
D112M3	*Random regression model that considers three regressors for genetic additive and permanent environmental effect at AOPU and THI, considering THI about 112 days.*

D112M4	*Random regression model that considers four regressors for genetic additive and permanent environmental effect at AOPU and THI, considering THI about 112 days.*
EBV	*Estimated breeding value*
EBV20_80	*Estimated breeding value in 20 months at temperature-humidity index equal 80*
IETS	*International Embryo Technology Society's*
ITU	Índice de temperatura e umidade
IOPU	*Interval between ovum pick-up*
HS	*Heat stress*
LIN	*Linear model (untransformed variable)*
LOG	*Logarithm model (transformed by logarithm)*
MOET	*Multiple ovulation embryo transfer*
MPP	*Model posterior probabilities*
MRA	Modelo de regressão aleatória
MSE	*Mean square error*
N	*Number*
NOPU	*Number of ovum pick-up*
NRC	*National research council*
P	*Permanent environment effect*
PEV	*Prediction error variance*
POI	*Poisson model*
PIVE	Produção *in vitro* de embriões
R	*Repeatability*
RRM	*Random regression models*
RH	*Relative humidity*
SD	Standard deviation
SM2	*Random regression model that considers two regressors for genetic additive and two regressors for permanent environmental.*
SM3	*Random regression model that considers three regressors for genetic additive and three regressors for permanent environmental.*

SM4	*Random regression model that considers four regressors for genetic additive and four regressors for permanent environmental.*
SS	*Service Sire*
TO	*Total oocytes*
THI	*Temperature-humidity index*
VE	*Viable embryos*
VO	*Viable oocytes*
1G1P	*Repeatability model*
1G2P	*Random regression model that considers one regressor for genetic additive and two regressors for permanent environmental.*
1G3P	*Random regression model that considers one regressor for genetic additive and three regressors for permanent environmental.*
1G4P	*Random regression model that considers one regressor for genetic additive and four regressors for permanent environmental.*
2G1P	*Random regression model that considers two regressors for genetic additive and one regressor for permanent environmental.*
2G2P	*Random regression model that considers two regressors for genetic additive and two regressors for permanent environmental.*
2G3P	*Random regression model that considers two regressors for genetic additive and three regressors for permanent environmental.*
2G4P	*Random regression model that considers two regressors for genetic additive and four regressors for permanent environmental.*
3G1P	*Random regression model that considers three regressors for genetic additive and one regressor for permanent environmental.*
3G2P	*Random regression model that considers three regressors for genetic additive and two regressors for permanent environmental.*
3G3P	*Random regression model that considers three regressors for genetic additive and three regressors for permanent environmental.*
3G4P	*Random regression model that considers three regressors for genetic additive and four regressors for permanent environmental.*
4G1P	*Random regression model that considers four regressors for genetic additive and one regressor for permanent environmental.*

4G2P	*Random regression model that considers four regressors for genetic additive and two regressors for permanent environmental.*
4G3P	*Random regression model that considers four regressors for genetic additive and three regressors for permanent environmental.*
4G4P	*Random regression model that considers four regressors for genetic additive and four regressors for permanent environmental.*

LISTA DE SÍMBOLOS

c^2	*Fraction of the phenotypic variance explained by the permanent environment*
h^2	*Heritability*
σ	Sigma
σ_u^2	*Additive genetic variance*
σ_{pe}^2	*Permanent environmental variance*
$\sum$	Somatório
β	Beta
δ	Delta
ρ	*Spearman rank correlation*
φ	Fi
$\times$	Multiplicação
$\otimes$	Kronecker product
G	*Covariance matrices genetics of the random regression coefficients*
P	*Covariance matrices permanent environmental of the random regression coefficients.*
A	*Kinship matrix*
I	*Identity matrix*
$\boldsymbol{E} = \sigma_{\boldsymbol{e}}^2$	*Residual matrix*

CAPÍTULO I

1. Introdução

A introdução do uso de técnicas embrionárias em animais domésticos iniciou-se no final da década de 1940, com o desenvolvimento de protocolos para superovulação e transferência de embriões, e se intensificou nos últimos 20 anos. Por exemplo, a produção mundial de embriões transferíveis saltou de entorno de 800 mil em 2000 para cerca 1.4 milhões em 2019, sendo produzidos 1.419.336 embriões transferíveis e, desse total, 72.7% foram produzidos *in vitro* (Viana, 2020). Atualmente, a indústria mundial de embriões bovinos é caracterizada por tendências divergentes: uma diminuição na produção e na transferência de embriões *in vivo*, contrastando com um aumento no número de transferências de embriões *in vitro* (Viana, 2020).

A produção de embriões *in vitro* (PIVE) é uma tecnologia reprodutiva usada para aumentar o número de progênies de fêmeas de alto valor genético (PARKER GADDIS et al., 2017). Nesse contexto, é importante destacar que somente essas fêmeas com alto valor genético, em razão de ser uma característica importante economicamente, são submetidas a essa tecnologia. Trata-se de um processo similar à inseminação artificial, que facilita a disseminação do material genético dos machos. Além do desenvolvimento da tecnologia de PIVE muitos esforços foram realizados para melhorar a eficiência da produção de embriões, entretanto, todos eles focados em fatores não genéticos (MERTON et al., 2009).

A utilização das técnicas embrionárias tem alto custo e grande variabilidade nos resultado (JATON et al., 2016a). Caso parte dessa variabilidade tenha origem genética aditiva, a seleção de animais pode ser usada para melhorar a resposta à seleção nos rebanhos (PARKER GADDIS et al., 2017; DE VRIES; KANIYAMATTAM, 2020). Conhecendo esse potencial, o criador poderá decidir quais animais serão utilizados nesse procedimento (JATON et al., 2016a).

Nesse sentido, as primeiras pesquisas realizadas na raça Holandesa no Brasil estimaram herdabilidade e repetibilidade para o número de embriões de 0,03 e de 0,13, respectivamente (TONHATI; LÔBO; OLIVEIRA, 1998). Mais recentemente, foram obtidas estimativas de herdabilidade de 0,17 e 0,14 para número de embriões totais viáveis na população de animais da raça Holandesa no Canadá (JATON et al., 2016a). Dessa forma, os autores concluíram que é possível aumentar a produção de embriões das vacas por meio de seleção. Além disso, a seleção de fêmeas com maior valor genético para produção de embriões poderia ser utilizada como uma ferramenta adicional disponível para os criadores identificarem animais *outliers* antes de tomar a decisão da escolha de uma possível doadora.

De forma semelhante aos estudos realizados para estimar a herdabilidade para produção de embriões, em 2016, foi estimada herdabilidade de 0.19 para número de oócitos viáveis mensurados em animais da raça Guzerá no Brasil (PEREZ et al., 2016). No mesmo trabalho também é reportada correlação genética positiva e de alta magnitude entre número de oócitos e embriões viáveis (PEREZ et al., 2016). Sendo assim, essas características podem ser utilizadas como critério de seleção em programas de melhoramento genético com objetivo de melhorar o desempenho dos animais nas biotecnologias reprodutivas.

É importante destacar que as avaliações genéticas para as características de embriões e oócitos são, geralmente, realizadas pelo uso do modelo de repetibilidade (ASADA; TERAWAKI, 2002; JATON et al., 2016a; MERTON et al., 2009; PEREZ et al., 2016; TONHATI; LÔBO; OLIVEIRA, 1998). Nesses modelos, as correlações genéticas e de ambiente permanente entre as medidas repetidas do mesmo animal ao longo de tempo animal são consideradas perfeitas (ρ=1). Portanto, não se considera que possam ocorrer mudanças na expressão da característica ao longo do tempo. Entretanto, isso pode não ser caso, como, por exemplo, a idade das fêmeas pode resultar em mudanças na sua fertilidade e, com isso, espera-se que a correlação entre a produção de oócitos aos dois e aos oito anos de idade pode ser diferente de um. Uma alternativa eficiente para modelar esse efeito seria os modelos de regressão aleatória (RRM), uma vez que esses assumem que a correlação genética e ambiental permanente entre medidas repetidas ao longo do tempo pode ser diferente de um (OLIVEIRA et al., 2019b).

Outro ponto a ser observado nas avaliações genéticas para produção de oócitos e embriões é o fato de que essas características são contagens ou percentuais que normalemente não apresentam distribuição normal dos resíduos. Essa limitação pode ser corrigida por meio da tranformação dos dados, por exemplo, pela transformação Logarítmica ou Anscombe (JATON et al., 2016a) ou a utilização de modelos lineares mistos generalizados com distribuições alternativas que melhor se ajustam à contagem de oócitos e embriões. A distribuição de Poisson apresentou melhores resultados com maior estimativa de herdabilidade e maior acurácia dos valores genéticos que as transformações dos dados (PEREZ et al., 2017a), sendo uma alternativa interessante para avaliação genética de animais da raça Guzerá.

Além dos fatores genéticos, o estresse por calor também pode afetar a qualidade do oócito ou embrião e comprometer a fertilidade no gado leiteiro. O estresse por calor já foi associado a reduções na taxa de fertilização (SARTORI et al., 2002). Isso porque o estresse por calor influencia negativamente o desenvolvimento embrionário e o processo de ovogênese, uma vez que a capacidade de o oócito ser fertilizado e se desenvolver até o estágio de blastócito é menor no verão em comparação ao inverno (HANSEN, 2013).

Uma forma de medir e quantificar o estresse térmico é através do índice de temperatura-umidade (ITU), que foi utilizado para determinar a tolerância ao calor em animais da raça Gir por Santana et al. (2015). Esses autores demonstraram que a seleção para produção de leite sem levar em consideração o componente genético que influencia a tolerância ao calor pode causar perdas em produtividade.

Diante do contexto, foram desenvolvidos três estudos. O primeiro utilizou modelos de regressão aleatória para avaliar o efeito da idade das fêmeas nas características de produção de oócitos e embriões. O segundo avaliou o efeito do estresse térmico por meio do índice ITU sobre as características de produção de oócitos e embriões utilizando modelos de regressão aleatória. O terceiro abordou à utilização de modelos lineares mistos generalizados e transformações dos dados na estimação de parâmetros genéticos para as características de produção de oócitos e embriões.

2. Revisão bibliográfica

2.1 Produção de embriões *in vivo, in vitro* e transferência de embriões

A transferência de embriões produzidos *in vivo* é uma biotecnologia reprodutiva na qual os embriões são coletados das trompas uterinas de uma fêmea doadora e depois são transferidos para o útero de uma outra fêmea, que é chamada de receptora e serve como mãe substituta para o restante da gestação (GADISA; FURGASA; DUGUMA, 2019). Essa técnica já foi aplicada em várias espécies de animais domésticos e também em animais selvagens e exóticos (GADISA; FURGASA; DUGUMA, 2019).

Em bovinos, a coleta de embriões foi realizada pela primeira vez de forma cirúrgica, um procedimento invasivo, caro, demorado e trabalhoso para as pessoas envolvidas, que exigia instalações cirúrgicas sofisticadas e não poderia ser realizada na fazenda de forma pratica. Além disso, as repetidas recuperações cirúrgicas no mesmo animal podem resultar no desenvolvimento de lesões graves nos ovários e fímbrias. Dessa forma, o surgimento de técnicas não-cirúrgicas beneficiou o procedimento, resultando em ampla disseminação e aplicação dessa técnica, em razão de seu fácil acesso, tanto para os técnicos quanto para os animais, e menor custo (MOORE; HASLER, 2017). Para a transferência de embriões ser realizada, é preciso de uma série de processos, entre eles: superovulação, inseminação de doadoras, coleta de embriões, isolamento, avaliação e armazenamento de curto prazo de embriões, micromanipulação e testes genéticos, congelamento de embriões e posterior transferência (BETTERIDGE, 2003).

O tratamento de animais doadores com gonadotrofina coriônica equina e o uso de prostaglandinas e seu análogo (cloprostenol) têm contribuído para a superovulação, resultando em um grande número de embriões (GADISA; FURGASA; DUGUMA, 2019) e fazendo o número médio aumentar de cinco ovócitos viáveis para entorno de 20 em animais *Bos taurus*, entretanto, para *Bos indicus* não há consenso científico sobre esse resultado (FERRÉ et al., 2020).

A PIVE envolve a coleta de ovócitos de folículos (aspiração folicular) e um período de amadurecimento (maturação *in vitro*), quando são misturados com esperma capacitado (fertilização *in vitro*) e o zigoto é cultivado no período de oito a nove dias para obtenção de blastocistos para transferência ao útero da fêmea receptora (GADISA; FURGASA; DUGUMA, 2019). A eficiência da PIVE varia significativamente, mas um número razoável é o de quatro embriões transferíveis por coleta ocorrida a cada 14 dias (DE VRIES; KANIYAMATTAM, 2020).

A coleta de oócitos para a PIVE pode ser feita por diversas técnicas, mas a principal é a técnica de aspiração folicular transvaginal, a qual tem apresentado uma maior flexibilidade e sido a melhor opção para a recuperação de oócitos in vivo na espécie bovina (MELLO et al., 2016). Os oócitos são aspirados de um pool heterogêneo de folículos antrais, de 2 a 8 mm de tamanho, incluindo folículos de ondas foliculares ovulatórias e não ovulatórias, bem como folículos dominantes e subordinados nessas ondas (FERRÉ et al., 2020).

A principal vantagem da PIVE é maximizar a exploração do potencial genético de fêmeas bovinas, porque permite a interação entre o espermatozoide e o oócito fora do trato reprodutivo da fêmea, com a possibilidade de formação de um novo indivíduo (MELLO et al., 2016). Entre outras vantagens da PIVE, estão: gerar filhos de animais inférteis, filhos de novilhas jovens antes da idade reprodutiva, salvar a genética de animais terminalmente doentes e/ou feridos, uso de vários touros em um curto período de tempo e a utilização de oócitos derivados de matadouro para produção de embriões de pesquisa e/ou de baixo custo (HASLER J. F.; BARFIELD, 2021). Mas existem alguns problemas, como o tamanho dos bezerros, a diminuição da intensidade do trabalho de parto, o aumento na taxa de aborto, aumentos nas taxas de má formação congênita e aumento na taxa de morte perinatal, além de menor taxa de gravidez quando comparada com embriões produzidos *in vivo* (HASLER J. F.; BARFIELD, 2021).

Embora no Brasil a PIVE tenha atingido escala comercial, ainda existem algumas limitações, como a baixa taxa de blastocisto que implica no aumento do custo de cada embrião produzido (MELLO et al., 2016). Assim como o fato de algumas fêmeas produzirem poucos oócitos e de baixa qualidade. Adicionalmente, os embriões produzidos por essa técnica apresentam baixa resistência ao congelamento, devendo ser transferidos frescos para obter maior taxa de prenhez (MELLO et al., 2016). E o sucesso da superovulação e da recuperação de embriões em bovinos depende de vários fatores relacionados à doadora e ao ambiente, como clima, instalações, protocolos hormonais, técnico responsável pelo procedimento, entre outros. Por fim, ressalta-se que as melhorias alcançadas nessa técnica facilitaram a conveniência dos protocolos, o bem-estar animal e a facilidade de gestão no procedimento de coleta. O fator limitante mais significativo no sucesso da superovulação tem sido a imprevisibilidade, devido à alta variabilidade entre os indivíduos, e na resposta ovariana à estimulação de gonadotrofinas (MIKKOLA; TAPONEN, 2017).

2.2 Situação atual do mercado de embriões

As biotecnologias ligadas à reprodução artificial avançaram muito e, hoje, os embriões congelados podem ser transferidos para recipientes adequados por meio de técnicas de amplo acesso, assim como a inseminação artificial. Uma combinação de transferência de embriões usando vacas de alto valor genético, inseminadas com touros provados, parece ser o uso mais comum da transferência de embriões bovinos mundial (MAPLETOFT, 2013).

No Brasil, a partir do ano de 2005, a PIVE ultrapassou a produção *in vivo*, e em 2014 o país foi considerado o maior produtor mundial de embriões *in vitro* (MELLO et al., 2016). O Brasil, nos anos 1990, era considerado como referência regional na utilização dessa tecnologia e, em apenas duas décadas, tornou-se líder mundial na produção de embriões *in vitro*, confirmando previsões sobre a taxa de aplicação da técnica. O crescimento do uso da técnica também foi observado em diversos países nos anos subsequentes (GONÇALVES; VIANA, 2019). Em 2016, o número de embriões viáveis produzidos via PIVE ultrapassou o número de embriões transferíveis produzidos *in vivo*, com base em dados registrados pela International Embryo Technology Society (IETS). No entanto, esse evento histórico se baseia apenas nos dados declarados submetidos ao IETS por participantes voluntários e, como tal, provavelmente não inclui o número total de embriões produzidos *in vivo* e PIVE em todo o mundo, portanto, é possível que o evento real tenha ocorrido ainda mais cedo (FERRÉ et al., 2020).

O uso de tecnologias embrionárias no Brasil aumentou notavelmente 726,5% nos últimos 20 anos, mas ainda representa apenas 0,33% do número de vacas e novilhas em idade reprodutiva. O aumento no uso de transferência de embriões no período entre 1995 a 2015 nos rebanhos leiteiros brasileiros foi ainda maior, 2.261,7%. No entanto, isso representa apenas 0.48% das fêmeas aptas à transferência e que foram efetivamente coletadas (VIANA; FIGUEIREDO; SIQUEIRA, 2017). É importante destacar que essa proporção também é baixa para o uso de outras tecnologias reprodutivas, como a inseminação artificial com apenas 13,3% das fertilizações realizadas em bovinos (VIANA; FIGUEIREDO; SIQUEIRA, 2017).

O grande destaque brasileiro na PIVE se deve, principalmente, ao tamanho e às características do rebanho nacional, como o alto número de animais zebuínos, que têm maior produção de oócitos por coleta (MELLO et al., 2016).

A transferência comercial de embriões em bovinos tornou-se uma indústria bem estabelecida. Embora um número muito pequeno de descendentes (em proporção) via tecnologias reprodutivas seja gerado anualmente, seu impacto é grande devido ao alto valor genético desses animais. Essa contribuição parece ser mais marcante na pecuária leiteira mundial, em que a maior parte do sêmen usado atualmente vem de touros criados via transferências de embriões (MAPLETOFT, 2013).

2.3 Influência genética na produção de embriões

As pesquisas realizadas com PIVE buscam avanços no desempenho geral do processo, tais como melhoria nos métodos para estimulação ovariana, recuperação e maturação de ovócitos, fertilização, desenvolvimento, congelamento e transferência do embrião e estabelecimento da prenhez (FERRÉ et al., 2020). Assim, vários fatores, incluindo aqueles relacionados aos animais e aqueles que envolvem o ambiente e o manejo (técnico, protocolo hormonal, aparelho de ultrassonografia), podem contribuir para melhorar os resultados da transferência de embriões (FLEMING et al., 2018a).

Adicionalmente, resultados de pesquisas indicam que existe um componente genético para características relacionadas à produção de oócitos e embriões e que a seleção animal é possível (CORNELISSEN et al., 2017; JATON et al., 2016a; MERTON et al., 2009; PARKER GADDIS et al., 2017; PEREZ et al., 2016). Além disso, o componente genético para características de superovulação envolve algumas regiões genômicas (BTA 5, e no BTA19, *SEC14L1*) que são similares àquelas de outras características de fertilidade atualmente avaliadas (PARKER GADDIS et al., 2017).

Ainda, alguns estudos relataram herdabilidade para produção de embriões viáveis variando de 0,03 até 0,34 (ASADA; TERAWAKI, 2002; JATON et al., 2016a; KÖNIG et al., 2007a; MERTON et al., 2009; TONHATI; LÔBO; OLIVEIRA, 1998). Enquanto as estimativas de repetibilidade variam de 0,13 até 0,34 (ASADA; TERAWAKI, 2002; JATON et al., 2016a; TONHATI; LÔBO; OLIVEIRA, 1998). Esses resultados indicam que a seleção de características de produção de embriões poderia acarretar um progresso genético para essas características, já que as herdabilidades estimadas variaram de baixas a moderadas. As estimativas de parâmetros genéticos não apresentam valores muito discrepantes entre animais zebuínos e taurinos. Por exemplo, para embriões clivados na raça Guzerá a herdabilidade é de 0,23 (PEREZ et al., 2017b) e o mesmo valor foi relatado para a raça Holandesa por Jaton *et al.* (2016b).

A seleção genética de animais para esse tipo de característica permitirá que os criadores identifiquem doadoras com maior valor genético para produção de embriões e oócitos, para que as futuras gerações respondam melhor a procedimentos como a superovulação e a fertilização *in vitro*.

Algumas regiões do genoma associada a características de superovulação e genes de interesse foram investigadas e validadas, como BTA3, BTA8, BTA11, BTA19, BTA27, BTA28, e BTA19 (PARKER GADDIS et al., 2017). A adição de informações genômicas à informação de pedigree afetou pouco as estimativas de herdabilidade para número de oócitos (0,31) e número de embriões (0,22). No entanto, ocorreu uma diminuição no erro padrão dessas estimativas, demonstrando uma acurácia ligeiramente maior quando se utiliza a informação genômica (CORNELISSEN et al., 2017) em detrimento da utilização exclusiva de informação genealógica na avaliação genética das populações.

As características de PIVE estão relacionadas geneticamente com outras características produtivas e reprodutivas. Correlações desfavoráveis de baixa a média magnitude entre a PIVE e a produção de leite, gordura e proteína foram relatadas na raça Gir (VIZONÁ et al., 2020) e na raça Holandesa (JATON et al., 2016b). Além disso, correlações de baixa magnitude, negativas e positivas, foram relacionadas com a idade no primeiro parto em doadoras da raça Guzerá (PEREZ et al., 2016). Entretanto, mesmo com correlações baixas, a seleção para vacas de alto desempenho produtivo que produzem grande número de embriões viáveis é uma alternativa interessante a ser utilizada (JATON et al., 2016b), uma vez que na população podem existir animais com bons valores genéticos para ambas características.

A inclusão do número de oócitos como critério de seleção nos programas de melhoramento genético de vacas leiteiras pode resultar em melhor desempenho da produção embrionária *in vitro* e maior retorno econômico do setor produtivo devido à quantidade razoável de variação genética detectada para essas características (VIZONÁ et al., 2020). Todavia, devido à PIVE aumentar o número de descendentes de fêmeas com alto valor genético, é importante ter cuidado com parâmetros de genética populacional, uma vez que na raça Gir o número efetivo de fundadores e ancestrais na população atual demonstra a existência de gargalos no pedigree e indicam a necessidade de monitoramento da estrutura populacional (SANTANA et al., 2014).

2.4 Modelo de regressão aleatória

Algumas características relacionadas à produção animal são avaliadas como medidas repetidas no tempo, como é o caso da produção de oócitos e embriões. Ao avaliar uma característica que se repete ao longo do tempo, modelos de repetibilidade, geralmente, são empregados. Entretanto, esse tipo de modelo não considera que a variância genética, variância de ambiente permanente e variância residual podem mudar ao longo do período avaliado, dessa forma, considera-se que existe uma correlação perfeita (ρ=1) entre a mesma característica avaliada em diferentes períodos de tempo.

Todavia, há uma forma de avaliar medidas repetidas, considerando que a correlação pode ser diferente de um, através do modelo de regressão aleatória. Inicialmente proposto por Henderson (1982), o modelo de regressão aleatória teve sua primeira aplicação em dados de produção animal conduzida por Ptak & Schaeffer (1993), na análise dos registros de produção de leite no dia do controle. Essa primeira tentativa não foi um MRA, mas foi responsável pela forma geral ou curva média de lactação para vacas dentro do rebanho, com ano e estação semelhantes.

Após esse ensaio inicial, Schaeffer & Dekkers (1994) estenderam os coeficientes de regressão do modelo de regressão fixa para efeitos aleatórios de animais. Ao fazê-lo, eles foram capazes de explicar a forma média da curva de lactação dentro de um determinado rebanho, ano e estação, bem como elucidar o desvio da curva de lactação de cada animal individual a partir dessa forma média. Os autores também foram capazes de explanar a mudança na estrutura de correlação de registros repetidos em indivíduos ao longo do tempo. Fornecendo, assim, uma estrutura robusta para modelar trajetórias de características e realizar análises genéticas simultaneamente (CAMPBELL et al., 2019). Desse modo, não é necessário utilizar fatores de ajuste para uma determinada idade ou dias em lactação, porque esse ajuste já está incluído na matriz de delineamento (ALBUQUERQUE, 2004).

Toda a informação fenotípica disponível pode ser incluída na avaliação genética, pois o MRA não exige que os registros sejam medidos ao mesmo tempo em todos os indivíduos e também não requer número mínimo de registros por animal (SCHAEFFER, 2004).

O uso de polinômios ortogonais de Legendre no MRA oferece estabilidade numérica, reduzindo a correlação entre os coeficientes de regressão aleatória e o erro de computação (SCHAEFFER, 2004). E, após obter os coeficientes para efeitos genéticos aleatórios, os valores genéticos em qualquer ponto do tempo podem ser facilmente calculados (BABA et al., 2020).

O modelo de regressão aleatória estatisticamente é:

$$Y_{ijkl} = hys_i + \sum_{k=0}^{n} \emptyset_k(mo_t)\beta_{jk} + \sum_{k=0}^{n} \emptyset_k(mo_t)u_{jk} + \sum_{k=0}^{n} \emptyset_k(mo_t)\, pe_{jk} + e_{ijkl},$$

em que Y_{ijkl} é o vetor das informações da característica; hys_i é o efeito fixo; β_{jk} é o coeficiente de regressão fixa; u_{jk} e pe_{jk} são os coeficientes de regressão aleatória que descrevem os efeitos genéticos aditivos e de ambiente permanente sobre a performance do animal; $\emptyset_k(mo_t)$ é função matemática ou polinômio usado como coeficiente de regressão, e e_{ijkl} é o erro aleatório.

Para os quais se assume:

$$Var\begin{bmatrix} \boldsymbol{u} \\ \boldsymbol{pe} \\ \boldsymbol{e} \end{bmatrix} = \begin{bmatrix} \mathbf{A} \otimes \mathbf{G} & 0 & 0 \\ 0 & \boldsymbol{I} \otimes \boldsymbol{P} & 0 \\ 0 & 0 & \boldsymbol{E} \end{bmatrix},$$

G e ***P*** são as matrizes de covariância dos coeficientes de regressão aleatória; ***A*** é a matriz de parentesco; ***I*** é uma matriz identidade; ***E*** $= \sigma_e^2$ é uma matriz diagonal dos residuos; e ⨂ é o produto de Kronecker entre as matrizes.

O MRA tem sido considerado o método de escolha para avaliar geneticamente caracteres longitudinais em várias espécies, incluindo gado leiteiro, gado de corte, ovelhas, caprinos, cavalos, suínos, aves, codornas e peixes (DZOMBA et al., 2011; OLIVEIRA et al., 2019b). O modelo de regressão aleatória também está despertando o interesse da área genômica do melhoramento animal (KANG et al., 2018; OLIVEIRA et al., 2019a), dessa forma, a atualização do modelo de regressão aleatória na avaliação de características de produção artificial pode ser uma alternativa interessante a ser pesquisada.

2.5 Estresse térmico na produção de embriões

A alta temperatura e a umidade do ambiente de criação resultaram em um declínio acentuado na qualidade dos oócitos recuperados de vacas *Bos taurus* e diminuíram acentuadamente suas capacidades de desenvolvimento *in vitro*. Entretanto, em vacas *Bos indicus,* um alto percentual de oócitos recuperados, exibiram morfologia normal e produziram uma alta proporção de blastocistos, independentemente da variação natural da temperatura e da umidade entre as estações do ano (ROCHA et al. 1998).

O genótipo é um dos principais determinantes da resistência ao estresse térmico, e as raças de gado de origem *Bos indicus* são mais resistentes às condições tropicais, como temperatura e umidade elevadas, do que raças que evoluíram em clima temperado (TORRES-JÚNIOR et al., 2008). A maior resistência dos animais zebuínos ao calor pode ser associada à seleção natural que, provavelmente, aumentou a frequência de alguns alelos favoráveis à resistência ao estresse térmico em populações que evoluíram em climas quentes (HANSEN, 2020). Dessa forma, esses animais conseguem suportar melhor os efeitos do estresse térmico, sem comprometer a produção e a reprodução.

Uma das melhores formas para avaliar o estresse térmico é através de variáveis bioclimáticas ou índices que contemplem mais de uma variável. O índice mais utilizado é o ITU, que avalia os efeitos combinados da temperatura e da umidade do ar (NASCIMENTO et al., 2019). Existem diferentes fórmulas de cálculo do ITU, entretanto, a mais comum e amplamente utilizada é a do National Research Council: $ITU = [\ (1,8*T+32) - (0,55-(0,0055*UR)\ *(1,8*T-26))\ ]$; em que T = temperatura de bulbo seco (ºC); UR = umidade relativa do ar (%).

A principal vantagem do ITU é que os dados necessários podem ser facilmente obtidos na fazenda ou a partir de uma estação meteorológica próxima, enquanto os dados de radiação térmica recebidos pelo animal e a velocidade do vento são mais difíceis de registrar, porque dependem de equipamentos específicos, e os dados necessários, muitas vezes, não estão disponíveis publicamente (BOHMANOVA; MISZTAL; COLE, 2007a).

No entanto, níveis elevados de ITU têm um efeito negativo na retomada da atividade ovariana em *Bos indicus*, especialmente se ocorrer ITU alto durante o último trimestre da gestação (DÍAZ et al., 2020). O processo de ovogênese é longo e não está claro até que ponto o estresse térmico pode afetar tanto nas fases iniciais do crescimento folicular quanto no período de maturação (HANSEN, 2013).

O oócito adquire seu potencial de desenvolvimento de forma gradual durante o desenvolvimento folicular e, portanto, perturbações induzidas pelo estresse térmico no funcionamento folicular podem levar a uma redução da competência do oócito ao ser fertilizado (HANSEN, 2013; WOLFENSON; ROTH, 2019). A competência dos oócitos de serem fertilizados ou de se desenvolverem até o estágio de blastocisto é menor no verão do que no inverno (HANSEN, 2013).

Um experimento com vacas Gir sob um período de 28 dias de estresse térmico não observou efeito imediato sobre a função reprodutiva, mas verificou que exerceu um efeito deletério tardio sobre o crescimento folicular ovariano, concentrações hormonais e redução da competência oocitária para fertilização *in vitro* em até 105 dias após o término do estresse térmico (TORRES-JÚNIOR et al., 2008). A falta de efeito imediato do estresse térmico na função reprodutiva sugeriu que a suscetibilidade dessa raça termotolerante necessita exposição a longo prazo a temperaturas elevadas.

A seleção somente baseada em características produtivas pode afetar negativamente a termotolerância. Santana et al. (2015) relataram que as tendências genéticas para a raça Gir indicam que no futuro poderá ser necessário selecionar animais termotolerantes para manter a produção alta mesmo em períodos de estresse térmico. O bem-estar animal também é comprometido pelo efeito do estresse térmico. Uma forma muito difundida avaliar o efeito do estresse térmico é através da quantificação da diminuição da produção de leite e da redução das taxas reprodutivas devido a sua facilidade de medição no nível do rebanho, tendo uma ligação direta com a lucratividade da fazenda (POLSKY; VON KEYSERLINGK, 2017). Esse tipo de medida é utilizado devido ao fato de que quantificar o bem-estar animal não é uma tarefa muito simples, dessa forma, reforçasse os efeitos negativos do estresse térmico sobre os animais (POLSKY; VON KEYSERLINGK, 2017). Por isso, investigar diferentes estratégias para mitigar o estresse térmico em diferentes espécies de animais deve ser uma prioridade devido às perspectivas das mudanças climáticas em nível global (HANSEN, 2013). Nesse contexto, a seleção de animais mais termotolerantes poderá ser uma alternativa viável de bons resultados.

2.6 Modelos lineares generalizados

As características de produção de oócitos e embriões são exemplos de atributos que apresentam distribuição não Gaussiana, assim como uma série de outras propriedades; e pela necessidade de fazer predições para este tipo de característica foram desenvolvidos os modelos lineares generalizados (MCCULLAGH; NELDER, 1983). Assim, a teoria do modelo linear generalizado permite que a variável y siga qualquer outra distribuição que pertença à família exponencial na forma canônica, ao invés de somente a distribuição normal (RESENDE; SILVA; AZEVEDO, 2014).

A transformação da variável dependente é uma alternativa a utilização de modelos lineares generalizados, dessa forma a variável passa apresentar uma distribuição normalizada e após isso, são realizadas as análises genéticas. Entre as transformações mais utilizadas para o número de oócitos e embriões estão a transformação logarítmica e a Anscombe (CORNELISSEN et al., 2017; JATON et al., 2016a; PARKER GADDIS et al., 2017; PEREZ et al., 2016). Entretanto, a transformação dos dados pode não ser a melhor opção para avaliação genética dessas características, Perez et al. (2017) relataram que, ao utilizar um modelo linear generalizado que seguia distribuição Poisson, foi possível capturar melhor a variância genética aditiva e de ambiente permanente, resultando assim em maiores estimativas de herdabilidade e repetibilidade. Os modelos lineares generalizados são uma extensão do modelo linear e incluem o conceito da “função de ligação”, que especifica a relação existente entre o valor esperado da variável y_i e o preditor linear η_i. Na função de ligação “Log”, $\eta_i = log_e\ (y_i)$ é comumente usada para modelagem de características de contagem (PEREZ, 2016). Essa particularidade é importante para análise de dados que não apresentam distribuição normal, como, por exemplo, variáveis binominais, escores e contagens. Devido a essa particularidade, não é necessário usar nenhum tipo de transformação dos dados para que eles tenham distribuição normal dos resíduos.

Alguns desses modelos já foram usados para características de interesse zootécnico, como contagem de carrapatos (AYRES et al., 2013), número de leitões nascidos vivos, número de leitões desmamados e mortalidade durante o período de amamentação (SILVA et al., 2019), número de oócitos viáveis, número de oócitos grau I, número de oócitos degenerados, número de embriões clivados e número de embriões produzidos viáveis (PEREZ et al., 2017a; VIZONÁ et al., 2020).

3. Hipóteses

1. As características de produção de oócitos e embriões apresentam variabilidade genética para serem utilizadas no processo de seleção.
2. A avaliação das características de produção de oócitos e embriões como medidas repetidas no tempo pode resultar em maior ganho genético do que o método tradicional como única medida.
3. As características de produção de oócitos e embriões sofrem influência da interação genótipo-ambiente, e são prejudicadas quando os animais são manejados em ambientes com altos valores de ITU.
4. Os componentes de variância das características de produção de oócitos e embriões sofrem alterações quando são utilizados diferentes métodos de transformação ou quando são usados modelos que consideram outros tipos de distribuição de probabilidade.

4. Objetivos

1. Estimar os componentes de (co)variâncias e parâmetros genéticos para as características de produção de oócitos e embriões.
2. Analisar as características de produção de oócitos e embriões como medida única ou como medida repetida no tempo.
3. Avaliar a influência da interação genótipo-ambiente sobre as características de produção de oócitos e embriões por meio de modelos de regressão aleatória, utilizando valores de ITU (ambiente) como efeito nos modelos.
4. Estimar os componentes de variância considerando diferentes transformações nos dados e/ou modelos que consideram diferentes distribuições de probabilidade.

CAPÍTULO II

Genetic evaluation of oocyte and embryo production in Dairy Gir cattle using repeatability and random regression models[3]

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[3] Article submitted in Revista Brasileira de Zootecnia

Genetic evaluation of oocyte and embryo production in Dairy Gir cattle using repeatability and random regression models

ABSTRACT

The objective of this work is to estimate genetic parameters and breeding values to improve embryo and oocyte production, using repeatability and random regression models (RRM) for Gir dairy cattle. We used 11,398 records of ovum pick-up from 1,747 Dairy Gir donors and evaluated sixteen different models: one is the traditional repeatability model and the other fifteen are RRM, each of which considered a different combination of Legendre polynomial regressors to describe the additive genetic and permanent environment effects. The 4G1P model (four regressors for the genetic effect and one regressor for the permanent environment effect) is the most suitable model to analyze the number of viable and total oocytes, while the 3G1P is the best model to analyze the number of cleaved and viable embryos. The heritability estimated using the RRM was higher than that estimated using the repeatability model. The high repeatability reported for oocyte and embryo count traits indicates that donors, which had high counts of oocytes and embryos in the first ovum pick-up, should maintain this result in the next ovum pick-up. Genetic correlations between adjacent ages were high and positive, while genetic correlations between extreme ages were weak. We observed a reordering of the top sires and females over the period evaluated. The reliability of the estimated breeding values by RRM showed changes across age, and the expected genetic gains by RRM are larger. This shows that RRM is

an efficient alternative for the evaluation and selection of oocyte and embryo count traits.

Keywords: animal breeding, *Bos Indicus*, dairy cattle, *in vitro* fertilization, ovum pick-up

1. Introduction

Selection for reproductive efficiency is an effective strategy to ensure the success of dairy and beef cattle industries (Watanabe et al., 2017). The use of reproductive biotechnologies began several decades ago, with the development of superovulation protocols and embryo transfer from the late 1940s. Reproductive technologies, including superovulation, *in vitro* fertilization, and embryo transfer are used to increase the reproductive rate of genetically superior females (Parker Gaddis et al., 2017).

Several factors, including those related to animals and those that involve environment and management, contribute to the low results of embryo transfer (Fleming et al., 2018). However, it is well-known that there is a genetic component related to superovulation traits and that selection should be applied to improve embryo transfer rates (Merton et al., 2009; Jaton et al., 2016a; Perez et al., 2017a, 2016; Parker Gaddis et al., 2017; Cornelissen et al., 2017).

Bos indicus show better results in *in vitro* oocyte and embryo production than *Bos taurus* (Lacerda et al., 2020). That is why the investigation of methodologies that consider the selection and improvement of the reproductive traits present a great demand for inclusion in genetic evaluations.

The genetic evaluation of embryo and oocyte traits is usually performed using repeatability models (Tonhati et al., 1998; Asada and Terawaki, 2002; Merton et al., 2009; Jaton et al., 2016a), in which the genetic and permanent environment correlations are assumed perfect(equal one); therefore, it is not considered that, over time, changes in genetic and permanent environment effects might occur.

However, random regression models (RRM) allow us to obtain individual estimated breeding value (EBV) curves and it also considers changes in genetic and residual variances over time, which can be applied to predict breeding values more accurately (Jamrozik and Schaeffer, 1997; Resende et al., 2001). In addition, it allows prediction of breeding values for any desired age, even for ages in which animal has not been recorded.

Considering the importance of Dairy Gir breed for dairy farming, this work aimed to estimate genetic parameters and breeding values for production of embryos and oocytes using repeatability and RRM to determine which model could result in greater genetic gains in oocyte and embryo production in dairy Gir animals.

2. Material and methods

2.1. Data

The data used were from three herds of Dairy Gir cattle, belonging to the same company, in the state of Minas Gerais – Brazil (19º55' S – 43º57' W). The complete data set considered for genetic analysis consists of 3,124 Gir cows that calved between 2000 and 2015, daughters of 250 sires.

The available information was on traits of total oocytes (TO), viable oocytes (VO), cleaved embryos (CE), and viable embryos (VE), resulting from 13,217 collection sessions, which produced a total of 60,092 viable embryos. The cows' age varies from 1 to 20 years at the time of ovum pick-up. The sessions were held between January 2005 and March 2017. The number of sessions of ovum pick-up varied from one to 53 per donor, and in 41.7% of cases, only one collection per donor was performed, and the intervals between sessions for the same donor varied from seven to 120 days.

For this study, we excluded records of females with extreme age at ovum pick-up (<12 or >144 months), number of TO equal to 0. For the other traits (VO, CE, and VE), the count records equal to 0 were kept and considered in the analyses. Only healthy animals with at least two individual records of ovum pick-up during age period were maintained. The contemporary groups for all traits are defined by the concatenation of herd, year, and ovum pick-up season. Seasons were classified into wet (October until March) or dry (April until September). Contemporary groups that contained fewer than five observations were eliminated.

Following these criteria, 11,398 records of ovum pick-up from 1,747 Dairy Gir heifers and cows from three farms, collected from 2010 to 2017, were analyzed. Semen from 212 different sires (Gir or Holstein) was used in *in vitro* fertilization procedures. We used the same database for all models evaluated. The pedigree file included 5,921 animals.

2.2. Statistical analyses

The statistical program R (R Core Team, 2017) was used for data editing and descriptive statistics. Data used in this work so they were transformed using the logarithmic transformation, e. g., total oocytes: TO = Ln (Total Oocytes + 1.001).

2.3. Genetic analyses

The estimates of variance components for ovum pick-up traits at age (months) were obtained using a random regression model. For all evaluated traits, the model considered contemporary groups, interval between collections, and number of collections as fixed effects. For VE, a fixed effect referring to sire used in artificial fertilization was added (Table 1).

Genetic parameters and breeding values were estimated using the model:

$$Y_{ijkl} = hys_i + iop_j + nop_j + \sum_{k=0}^{n} \emptyset_k(mo_t)u_{jk} + \sum_{k=0}^{n} \emptyset_k(mo_t)\, pe_{jk} + sa_l + e_{ijkl},$$

in which Y_{ijkl} is the vector of trait value (TO, VO, CE, and VE) in month t within the herd-year-season of ovum pick-up; hys_i is the fixed effect of herd-year-season of ovum pick-up; iop_j is the covariable effect of the interval between ovum pick-up in the same animal; nop_j is the covariable effect of animal's ovum pick-up number; sa_l fixed effect referring to sire used in artificial fertilization; u_{jk} and pe_{jk} are random regression coefficients that describe, respectively, the additive genetic and permanent environment effects on the performance of animal j; Øk(mo_t) is Legendre polynomial for registration in month of ovum pick-up of animal *j* in month *t*, in which *k* is Legendre polynomial coefficient; and e_{ijkl} is the random error.

For which we assume:

$$Var\begin{bmatrix} \boldsymbol{u} \\ \boldsymbol{pe} \\ \boldsymbol{e} \end{bmatrix} = \begin{bmatrix} \mathbf{A} \otimes \mathbf{G} & 0 & 0 \\ 0 & \boldsymbol{I} \otimes \boldsymbol{P} & 0 \\ 0 & 0 & \boldsymbol{E} \end{bmatrix},$$

in which ***G*** and ***P*** are the covariance matrices of the random regression coefficients, ***A*** is the kinship matrix, ***I*** is the identity matrix, ***E*** = $\sigma_{\boldsymbol{e}}^2$ is a diagonal (residual) matrix, and $\otimes$ is Kronecker product between the matrices.

The additive genetic effects and permanent environment effect were estimated using Legendre polynomials (Kirkpatrick et al., 1994):

$$y_t = \sum_{i=0}^{n} \alpha_i \Phi_i(m_t^*)$$

in which m_t^* is the standardized time unit (months) ranging from −1 to +1; $mo_t^* = -1 + \frac{2\,(mo_t - mo_{min})}{mo_{max} - mo_{min}}$, wherein, mo_{min} and mo_{max} are the age at ovum pick-up in months, the lowest (12 months) and the highest (144 months) reported in the data set after consistency edits, respectively; and m_t is the age at ovum pick-up.

For the standardized age at ovum pick-up, it can be defined as:

$$\emptyset(mo_t^*)k = \frac{1}{2^K}\sqrt{\frac{2K+1}{2}}\sum_{m=0}^{K/2}(-1)^m \binom{k}{m}\binom{2K+1}{r}(mo_t^*)^{r-2m}$$

in which mo_t^* is *i*-th age at ovum pick-up, *i* is the order of the Legendre function, and m is the number of indices needed to determine the polynomial. The residual variance (σ_e^2) was considered homogeneous for all functions.

To assess which model had the best fit to be used in the genetic evaluation of traits of artificial reproduction, we sought to adjust the genetic and permanent environment effects using different polynomial functions from the first to the fourth order, totaling 16 different combinations between genetic and permanent environment effects. The models design can be seen in Table 1, e. g., 1G1P is a traditional repeatability model and 4G3P is a random regression model that considers four regressors for genetic additive and three regressors for permanent environmental.

The estimated breeding value of animal i in month t was calculated as $EBV_{it} = \mathbf{z'}_t\hat{\alpha}_i = \sum_{j=0}^{k_a-1} \alpha_{ij}\, \emptyset_j(mo_t)$, in which i $\hat{\alpha}_i$ a vector (k_a × 1) of estimates of the additive genetic random regression coefficients of animal i and z_t a vector of Legendre polynomial coefficients for month t.

All analyzes were estimated through the maximum restricted likelihood method, using the program REMLF90 (Misztal et al., 2002). The quality of fit was carried out through comparison tests between models: Akaike's information criterion ($AIC = -2\log L{+}2p$, in which p is the number of parameters in the model) and Schwarz's Bayesian information criterion ($BIC = -2logL + p\ log(\lambda)$, in which log (λ) is natural logarithm of sample size (or dimension of y) and p is the number of parameters in the model).

In the heritability, $h2_{mo} = \frac{\sigma^2_{a(mo)}}{\sigma^2_{a(mo)}+\sigma^2_{pe(mo)}+\ \sigma^2_e}$, and repeatability, $R_{mo} = \frac{\sigma^2_{a(mo)}+\sigma^2_{pe(mo)}}{\sigma^2_{a(mo)}+\sigma^2_{pe(mo)}+\ \sigma^2_e}$, $\sigma^2_{a(mo)}$ and $\sigma^2_{pe(mo)}$ are additive genetic and permanent environmental variances for months (mo) and were calculated and plotted graphically to check for possible differences between the repeatability model (1G1P) and the best fit model selected by AIC and BIC criteria.

The genetic correlation between two months in ovum pick-up, mo_{t1} and mo_{t2}, was calculated by dividing the additive genetic covariance between months in ovum pick-up, mo_{t1} and mo_{t2}, by the product of the square root of the genetic variances of months in ovum pick-up mo_{t1} and mo_{t2}:

$$r_{g\ (mo1,mo2)} = \frac{cov_{g(mo1,mo2)}}{\sqrt{var_{g(mo1,mo1)} + var_{g(mo2,mo2)}}}$$

in which $cov_{g(mo1,mo2)}$ is the genetic covariance between mo_{t1} and mo_{t2} months, $var_{g(mo1,mo1)}$ and $var_{g(mo2,mo2)}$ are additive genetic variance of mo_{t1} and mo_{t2} months, respectively. The permanent environmental correlation was calculated in the same way as above, just replacing the genetic matrices with those of permanent environment.

To verify the occurrence of changes in the ranking of the best animals at different ages at ovum pick-up, Spearman's ranking correlation was calculated between the classifications of the 5% of the best sires based on estimated breeding values (EBV) by the 1G1P model and by the best model chosen by the AIC and BIC criteria. For the chosen model, the classification by the EBV at six different ages (24, 48, 72, 96, 120, and 144 months) was used. The same procedure was performed for the 5% best females.

The reliabilities were calculated using the triangular matrices of prediction error (co)variances for random regression effects, from the inverse of the mixed model equations obtained in BLUPF90 program (Misztal et al., 2002).

The expected genetic gain was calculated through: $\delta G_i = h_{mo}^2 * isel * \sigma_i$, in which δG_i is the genetic gain due to direct selection for trait i, h_i^2 is the heritability of trait i, $isel$ is the selection intensity, and σ_i is the phenotypic standard deviation after logarithmic transformation.

3. Results

3.1. Data

The number of oocytes and embryos varied widely (Table 2); for all traits; the median was less than the mean, and the standard deviation showed high values, in some cases greater than the median, and almost equal to the mean. The number ovum pick-up varied over the age period studied (Figure 1); most collections were made in young animals, from the month 72 onwards, and there was a drastic reduction in the number of collections.

3.2. criteria

The AIC indicated more than one model as the best (Table 3). However, the BIC, which is more discerning, indicated only one model for each trait. That said, the 4G1P model was chosen for the TO and VO traits, and for CE and VE the best fit model was the 3G1P. The models with a higher number of coefficients for permanent environmental effect did not show good results compared with models with a higher number of coefficients for additive genetic effect.

3.3. Heritability

The estimated genetic parameters varied according to the age at ovum pick-up (Figure 2). Using RRM, the highest values of heritability were estimated at the lowest and highest ages (extremes) at ovum pick-up. The h2 ranged from 0.46 (76 months) to 0.72 (144 months) for TO using 4G1P model and 0.37 using 1G1P model; for VO, it varied from 0.43 (84 months) to 0.66 (144 months) using 4G1P and 0.34 using 1G1P. In the case of CE, using 3G1P model, it ranged from 0.31 (36 months) to 0.62 (144 months) and 0.25 with 1G1P. For VE, h2 varied from 0.20 (96 months) to 0.40 (144 months) using 3G1P and 0.17 with 1G1P.

In summary, the 1G1P model produced the lowest heritability estimates for all traits. The RRM captured the highest proportions of additive genetic effects, producing the highest heritability values for all traits, especially in the initial and final evaluated periods, in which there is a smaller number of observations (Figure 1).

The highest h2 estimates were reported in the first months of ovum pick-up and after 84 months of age, in which, after that period, there is a linear increase in the heritability value.

3.4. Repeatability

There is little difference in repeatability (R) estimates between the months 24 and 96 for models 1G1P and RRM. In the initial period, 12 to 24 months, the R of models considering random regression for genetic effect was greater than the 1G1P model (Figure 4). From 96 months onwards, there was a linear increase in R until the end of the period.

For the TO, using the 1G1P model, the R was 0.54, while with the 4G1P model, R ranged from 0.54 (72 months) to 0.76 (144 months), with the highest values reported in the late age period and the lowest values in the intermediate period. The VO showed R of 0.51 considering 1G1P model and varied between 0.50 (84 months) and 0.70 (144 months), with the highest values also found in final period and the lowest values in the intermediate period.

The CE presented R of 0.42 for 1G1P model and varied between 0.41 (36 months) and 0.67 (144 months) for 3G1P, with the highest values also being found in final ages and lowest values in period between 24 to 48 months. The VE presented

R of 0.35 for 1G1P and ranged from 0.35 (96 months) to 0.51 (144 months) for 3G1P, with the highest values reported for age in last months (132-144 months) and the lowest values for intermediate ages.

3.5. Genetic and permanent environment correlation

The genetic correlations ranged from 0.98 to −0.24 for TO, 0.98 to −0.19 for VO, 0.99 to −0.03 for CE, and 0.99 to −0.04 for VE (Figure 4). Strong and positive correlations were reported between adjacent ages, and the lowest and negative correlations were observed between the extreme ages.

The models 4G1P and 3G1P indicated that only a linear coefficient (1P) is efficient to evaluate the effect of permanent environment, so the correlation of permanent environment is equal to 1, because no change in the value of variance of permanent environment was observed across ages.

3.6. Spearman's rank correlation

The magnitude of the estimated Spearman's rank correlations coefficient confirmed the rearrangement of the top 5% sires and females in most comparisons (Tables 4, 5, 6, and 7). Altogether, 168 rank correlations were calculated, and only five of these (2.97%) presented a value above 0.80. This demonstrates a reranking of the best animals when considering repeatability versus random regression over the evaluated age period (12-144 months) when using RRM.

Ranking correlation of 0.86 was reported among the top 5% best sires for the TO trait in the 4G1P_24 and 4G1P_120 (Table 4) models. For VO, the ranking correlation of 0.80 was reported between the top 5% best females ranked by the 1G1P model with those ranked at 48 months by the RRM (Table 5).

In the case of CE, no ranking correlation greater than 0.80 was reported (Table 6). For VE, ranking correlations of 0.84 and 0.85 were reported between 3G1P_24 and 3G1P_144 and 3G1P_48 and 3G1P_72 for the top 5% best sires, and a ranking correlation of 0.84 was estimated between 3G1P_48 and 3G1P_72 (Table 7) for the top 5% best females.

3.7. Reliability

The average reliability of EBV for TO of top 5% sires (Figure 5) using 1G1P model was 0.38, while reliability of EBV using the 4G1P model varied from 0.38 (72 months) to 0.15 (144 months); the highest reliability values were reported at intermediate ages (60 to 84 months). For the top 5% females (Figure 6), the average reliability of EBV of NO using 1G1P model was 0.61, while using the 4G1P model, it varied from 0.64 (48 months) to 0.20 (144 months); the highest reliability values were reported at the initial intermediate ages (36 to 60 months).

For VO, the average reliability of EBV of top 5% sires (Figure 5) using 1G1P was 0.34, while using the 4G1P, it ranged from 0.35 (72 months) to 0.13 (144 months); the highest values were reported at intermediate ages (48 to 72 months). In top 5% females (Figure 6), the average reliability using 1G1P model was 0.59 and using the 4G1P model, it varied from 0.63 (48 months) to 0.20 (144 months); the highest values were reported at intermediate ages (36 to 60 months).

In the case of CE, the average reliability of EBV for CE of top 5% sires (Figure 5) using the 1G1P model was 0.26, and using the 3G1P model, it ranged from 0.41

(120 months) to 0.20 (36 months); the highest values were reported at advanced ages (96 to 132 months). The average reliability of EBV for CE of top 5% females (Figure 6) using 1G1P model was 0.51, and using the 3G1P model, it varied between 0.52 (96 months) for 0.41 (12 months); the highest values were reported in the final intermediate ages (84 to 108 months).

In relation to VE, the average reliability of EBV of top 5% sires (Figure 5) using the 1G1P model was 0.20, and using the 3G1P model, it ranged from 0.20 (72 months) to 0.07 (144 months); the highest values were reported at intermediate ages (48 to 84 months). In the case of top 5% females (Figure 6), the average reliability of EBV using 1G1P model was 0.42, and using the 3G1P model it varied between 0.44 (48 months) and 0.16 (144 months); the highest values were reported at intermediate ages (48 to 72 months).

3.8. Expected genetic gain

When comparing the 4G1P and 3G1P models to 1G1P (Figure 7), the expected genetic gain was greater throughout the age period evaluated, and the greatest difference was found in the initial and final periods.

The difference between the expected genetic gains for TO obtained by 4G1P and 1G1P models were at least 0.05 (72 months) and maximum value of 0.23 (144 months), and in case of VO, this difference between expected genetic gain obtained by the cited models was at least 0.05 (84 months) and maximum of 0.22 (144 months).

When comparing the expected genetic gain for CE and VE, the difference was at least 0.04 (36 months) and 0.02 (96 months), and maximum of 0.26 (144 months) and 0.17 (144 months), for CE and VE, respectively.

4. Discussion

4.1. Data

In all traits, the mean value was higher than the median, indicating that there are animals with high counts and that, thereby, could increase the mean value. The high standard deviation values, in some cases higher than the median, indicate that there is great variance in these traits.

The performance of *Bos indicus* and *Bos taurus* cows under *in vitro* embryo production technology has been reported in the literature, and the results indicate that there are significant differences between genetic groups (Vizoná et al., 2020). However, there is no obvious biological explanation for the greater number of oocytes recovered in *Bos indicus* (Pontes et al., 2011). Based on this information, we compared the phenotypic values reported in this work only with those of *Bos indicus* animals.

For VO, the values of our work are higher than the reports of 15.6 ± 12.7 by Perez et al. (2017b) for the Guzerá breed, and of 12.39 ± 10.04 by Vizoná et al. (2020) for Dairy Gir breed. Results for VE are similar to those of Vizoná et al. (2020), 4.59 ± 4.64, and lower than the values of Perez et al. (2017b), 6.1 ± 5.7. For the CE trait, values of 12.2 ± 10.0 (Perez et al., 2017b) are similar as the mean, but with greater standard deviation. None of these authors worked with the number of total oocytes, but it is likely that this value would be a little higher than the value of the number of viable oocytes, both on average and in standard deviation. In general, these reported differences are not of great magnitude.

4.2. Selection criteria

Among the sixteen models tested, only two of them were best for fit using AIC and BIC tests, thus forming two pairs of traits evaluated by each model, TO and VO by the 4G1P and CE model and VE by the 3G1P model, which indicates subtle differences between traits, that is, only one more degree to adjust the genetic effect.

More parameterized models usually adapt better to the data. However, this greater parameterization increases the computational demands and often does not substantially alter the result. The use of criteria that penalize more parameterized models and that give preference to more parsimonious models is indicated. In the case of traits studied in this paper, the use of a greater number of regressors for the additive genetic effect was indicated. In other traits, such as milk yield, some studies point to the need to use a greater number of coefficients to model the permanent environmental effect, compared with additive genetic effect (Canaza-Cayo et al., 2015).

4.3. Heritability

For all traits, using RRM, the h2 curve along the age presented a “U” shape. Usually, only a few records were collected at extreme ages as presented in Figure 1, and higher values of h2 at the beginning and end of the curve may be related to the number of records (Meyer, 1999).

Heritability for TO reported in the literature ranges from 0.13 in Holstein cattle raised in United States (Parker Gaddis et al., 2017) to 0.31 also in Holstein raised in the Netherlands (Cornelissen et al., 2017). These values are below those reported in this study, for both 1G1P and 4G1P models, indicating that in the Gir herd, this trait will respond more quickly to selection than in Holstein herds. The use of RRM (4G1P) could also respond more quickly to the selection than the repeatability model (1G1P).

For VO, h2 ranges from 0.16 to 0.25 in Brazilian Guzerá Breed depending on the type of distribution used (Perez et al., 2017b). Using a Bayesian model with Poisson distribution, Vizoná et al. (2020) reported h2 of 0.32. In studies using information from MOET (multiple ovulation and embryo transfer), e.g. Merton et al. (2009) and Perez et al. (2017a), there can be a pre-selection of animals that will be subjected to collection, which can result in lower heritabilities (Vizoná et al., 2020).

In the case of CE, h2 varies between 0.10 (Parker Gaddis et al., 2017) and 0.23 (Perez et al., 2017b; Jaton et al. 2016), and in the case of viable embryos, it varies from 0.10 (Perez et al., 2016) to 0.56 (Peixoto et al., 2004). According to Merton et al. (2009) and Cornelissen et al. (2017), the heritability for TO is higher than the heritability for CE and VE. Comparing the estimates between the traits, the VE has the lowest heritability value; this must be because this trait has a large number of non-genetic factors that can influence the result and affect service sire (Vizoná et al., 2020).

The higher heritability values recorded for the population of this study, compared with most of the works reported in the literature, are probably related to the fact that the farms present similar conditions, are located in the same region, and have the same technical support and animal husbandry techniques, which standardizes management and highlights genetic differences between animals.

4.4. Repeatability

The R estimates were similar between the models for each trait in intermediate ages. The highest values recorded in the initial and final periods are due to the higher estimates of the genetic variance in these months, which is related to the ability of the Legendre polynomials to make predictions in the ends of the curve and the number of ovum pick-up in that period.

The R for TO in intermediate ages at measurement was close to that reported by Parker Gaddis et al. (2017) for Holstein donors, but in general, it was higher than that reported by other works in the literature. The R values for VO, except in the final period, was close to that reported by Perez et al. (2017b) for Guzerá breed using a Bayesian model that considered Poisson distribution for data.

The R for CE was also within the values reported in the literature, except after the 120-month age at ovum pick-up. Perez et al. (2017b) reported R of 0.60 for this trait when using a Bayesian model, and Peixoto et al. (2004) reported R of 0.47 for donors of Nelore breed when using untransformed data.

For these traits, these R estimates can be explained by the high repeatability of the number of follicles per follicular wave (Jaton et al., 2016b). A study on the average number of follicles per single donor wave reported an extremely high R of 0.89 (Ireland et al., 2007). Due to the dependency relationship between the production of embryos and the number of follicles, it makes sense that the number of embryos per procedure can also be repeated in a donor (Jaton et al., 2016b).

The R is a useful tool for quantifying the extent to which an individual's performance remains consistent over time. The high R reported for oocyte and embryo count traits indicates that donors, which had high counts of oocytes and embryos in the first ovum pick-up, should maintain this result in the next ovum pick-up. Therefore, donors can be selected from after the first ovum pick-up, with special attention to puncturing young females (even in pre-puberty); this procedure should give good indications of life potential of these animals (Vizoná et al., 2020).

4.5. Genetic and permanent environment correlation

Positive and high genetic correlations indicate that the selection to increase oocyte or embryo production in each age at measurement may have a positive influence on oocyte and embryo production in another age at ovum pick-up.

The oocyte and embryo production in ages at ovum pick-up between 84 and 96 months was positively correlated with all the others, in some cases even medium and weak, indicating that this period would be the best one to select due to the correlated response in other periods. However, to accelerate the genetic gain, the selection should be carried out in ages at ovum pick-up under 12 to 48 months, prioritizing genetic gains at the beginning of the cow's productive life.

In distant periods in time, such as 12 and 144 months, low correlations have been reported, and in some cases even negative, such as −0.19 for OT at 12 months and 144 months. These correlation values are not expected in biological terms, but have already been reported for other traits and have been attributed to the poor fit of the RRM using Legendre polynomials at the extreme points of the curve, which is probably associated with the lower number of observations during these periods (Meyer, 1999).

In Brazilian Dairy Gir cattle, negative genetic correlations between the initial and final periods of lactation were reported by Pereira et al. (2010) and Pereira et al. (2013), both studies reporting that these correlations are not biologically expected. Results like this may be related to a pre-selection carried out in which animals had registered productions. Kern et al. (2018) reported that within a herd, not all animals are evaluated for type traits in Brazil, so some type of bias can be found in genetic evaluation. Another important factor is the extent of the period evaluated for counting oocytes and embryos, 132 months, which is a much longer period than the lactation period, which is usually 305 days (or 10 months). This long period can explain, in part, these correlations, but it makes it difficult to comparisons with small periods. However, this large period that has been evaluated demonstrates that there is considerable variation over time, especially when it comes to the genetic component that affects these traits, and this variation should not be overlooked.

4.6. Spearman's rank correlation

Livestock decision-making depends mainly on the level of genetic superiority of the animal, and the correlation between the EBV of different models is an important issue to be considered in an animal breeding program (Perez et al., 2017b).

The differences in magnitude of rank correlations between EBV can be explained, mainly, by the approach of each methodology in modeling productions in each measure (El Faro and Albuquerque, 2005). Herrera et al. (2008) comparing repeatability model with RRM, reported a ranking correlation of 0.74 for all animals and 0.65 for sires with progeny for 305-day milk yield in Gir dairy cattle.

In Guzerá herds, Santos et al. (2014) reported moderate to high correlations, indicating the possibility of differences in the ranking of animals, once RRM are adopted in place of the conventional model used in the genetic evaluations for milk yield. Variations in ranking correlations are probably due to differences in data consistency requirements, the functions used to adjust random effects, and the fixed effects included in random regression models (Pereira et al., 2019).

Alteration between the rankings of the best animals with the variation in age of ovum pick-up indicates that there is no well-defined relationship between the genes that influence oocyte and embryo production throughout the female's life. For this reason, changes in the choice of sires and donors in different periods must happen, since high selection intensities are used in this case. It is necessary to define only a period on which the selection should be based; the best would be right after puberty to improve these traits in young animals and to shorten the generation interval.

4.7. Reliability

The reliability estimated by the 4G1P and 3G1P models, in some periods, was higher than that estimated by the 1G1P model. In general, for TO, VO, and VE, the highest reliability values were reported for intermediate periods; in the initial and final periods, the reliability of these selected animals was lower, which may be related to the amount of collections that is higher in the intermediate period. In contrast, the reliability for the EBV of CE was higher in the interim period until the end.

The replacement of the repeatability model with RRM resulted in an increase in reliability of the EBV for 305-day milk yield (Padilha et al., 2016). In our study, the gains in reliability occurred in the period when the greatest number of collections occurs. In the initial and final parts of the evaluated period, the reliability estimated by the 4G1P and 3G1P models was lower than that estimated by the 1G1P model. Increasing the number of collections (Figure 1) is related to reliability (Figures 5 and 6); therefore, increasing the number of collections in young animals favors the EBV reliability at an early age.

4.8. Expected genetic gain

Although the results indicate that the selection using the 4G1P and 3G1P models would be more efficient to improve of oocyte and embryo production in Gir dairy breed, mainly in young animals, the lower reliability of the EBV in the initial period may be a factor limiting the use of these models.

The increase in the number of ovum pick-up in young animals is recommended, due to the possibility that reliability is associated with the number of ovum pick-up, which, in future evaluations, using RRM could result in increased EBV reliability in the initial period.

5. Conclusions

The use of a more accurate model that allows an increase in productive gains is of great importance. In this sense, the use of the RRM to replace the repeatability model in the genetic evaluation of total oocytes, viable oocytes, cleaved embryos, and viable embryos increases the genetic gains in these traits in dairy animals; the same can be expected in other breeds.

These traits have high repeatability values, indicating that the same counts must be repeated in different observations for the selection of animals after the first ovum pick-up with precision.

The reliability of the EBV by the RRM is greater in the periods when the greatest number of ovum pick-up occurs; therefore, increasing ovum pick-up in young animals increases the EBV reliability at an early age.

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Table 1 - Model design

Trait	Fixed effect				Regressor		Model
	CG	IVOP	NVOP	Sire	A	P	
TO, VO, CE	*	*	*	-	+	+	1G1P
	*	*	*	-	+	+ +	1G2P
	*	*	*	-	+	+ + +	1G3P
	*	*	*	-	+	+ + + +	1G4P
	*	*	*	-	+ +	+	2G1P
	*	*	*	-	+ +	+ +	2G2P
	*	*	*	-	+ +	+ + +	2G3P
	*	*	*	-	+ +	+ + + +	2G4P
	*	*	*	-	+ + +	+	3G1P
	*	*	*	-	+ + +	+ +	3G2P
	*	*	*	-	+ + +	+ + +	3G3P
	*	*	*	-	+ + +	+ + + +	3G4P
	*	*	*	-	+ + + +	+	4G1P
	*	*	*	-	+ + + +	+ +	4G2P
	*	*	*	-	+ + + +	+ + +	4G3P
	*	*	*	-	+ + + +	+ + + +	4G4P
VE	*	*	*	*	+	+	1G1P
	*	*	*	*	+	+ +	1G2P
	*	*	*	*	+	+ + +	1G3P
	*	*	*	*	+	+ + + +	1G4P
	*	*	*	*	+ +	+	2G1P
	*	*	*	*	+ +	+ +	2G2P
	*	*	*	*	+ +	+ + +	2G3P
	*	*	*	*	+ +	+ + + +	2G4P
	*	*	*	*	+ + +	+	3G1P
	*	*	*	*	+ + +	+ +	3G2P

*	*	*	*	+ + +	+ + +	3G3P
*	*	*	*	+ + +	+ + + +	3G4P
*	*	*	*	+ + + +	+	4G1P
*	*	*	*	+ + + +	+ +	4G2P
*	*	*	*	+ + + +	+ + +	4G3P
*	*	*	*	+ + + +	+ + + +	4G4P

TO - total oocytes; VO - viable oocytes; CE - cleaved embryos; VE - viable embryos.

CG = Contemporary group, IVOP = Interval between ovum pick-up, NVOP = Number of ovum pick-up.

* Considered; -: not considered; +: order of the Legendre polynomial.

Table 2 - Number of observations (N), medians, means, standard deviations (SD), minimum (Min) and maximum (Max) (untransformed variables)

Trait	N	Median	Mean	SD	Min	Max
Total oocytes	11398	13.00	21.72	15.38	1.00	182.00
Viable oocytes	11398	13.00	16.24	13.01	0.00	182.00
Cleaved embryos	11398	10.00	11.99	8.87	0.00	98.00
Viable embryos	11398	3.00	4.55	4.53	0.00	43.00

Figure 1 - Frequency of ovum pick-up in the age period evaluated.

Table 3 - Selection criteria based on values of goodness-of-fit tests obtained by repeatability and random regression models in Brazilian dairy Gir cattle

Model	Total oocytes		Viable oocytes		Cleaved embryos		Viable embryos	
	AIC	BIC	AIC	BIC	AIC	BIC	AIC	BIC
1G1P	**−1,450**	**−1,444**	**1,461**	**1,467**	**2,023**	**2,030**	**7,036**	**7,043**
1G2P	−1,623	−1,613	1,327	1,337	1,945	1,955	7,019	7,029
1G3P	−1,648	−1,632	1,306	1,322	1,928	1,944	7,006	7,022
1G4P	−1,673	−1,648	1,288	1,313	1,920	1,945	7,005	7,030
2G1P	−1,717	−1,707	1,257	1,267	1,899	1,909	7,014	7,024
2G2P	−1,720	−1,705	1,256	1,270	1,899	1,913	7,016	7,030
2G3P	−1,743	−1,722	1,235	1,256	1,883	1,904	7,002	7,023
2G4P	−1,761	−1,733	1,222	1,251	1,878	1,907	7,001	7,030
3G1P	−1,764	−1,748	1,213	1,230	**1,876**	**1,892**	**7,001**	**7,017**
3G2P	−1,764	−1,743	1,215	1,236	1,878	1,899	7,003	7,023
3G3P	−1,764	−1,738	1,214	1,241	1,879	1,906	7,001	7,028
3G4P	−1,774	−1,739	1,208	1,243	1,875	1,910	7,000	7,035
4G1P	**−1,782**	**−1,758**	**1,200**	**1,225**	1,870	1,895	7,001	7,026
4G2P	−1,782	−1,753	1,201	1,230	1,871	1,900	7,004	7,033
4G3P	−1,782	−1,747	1,200	1,235	1,873	1,908	7,003	7,037
4G4P	−1,777	−1,734	1,204	1,247	1,874	1,918	7,003	7,046

AIC - Akaike's information criterion; BIC - Bayesian information criterion.

In bold, the lowest values of AIC and BIC, and the values of the repeatability model (1G1P).

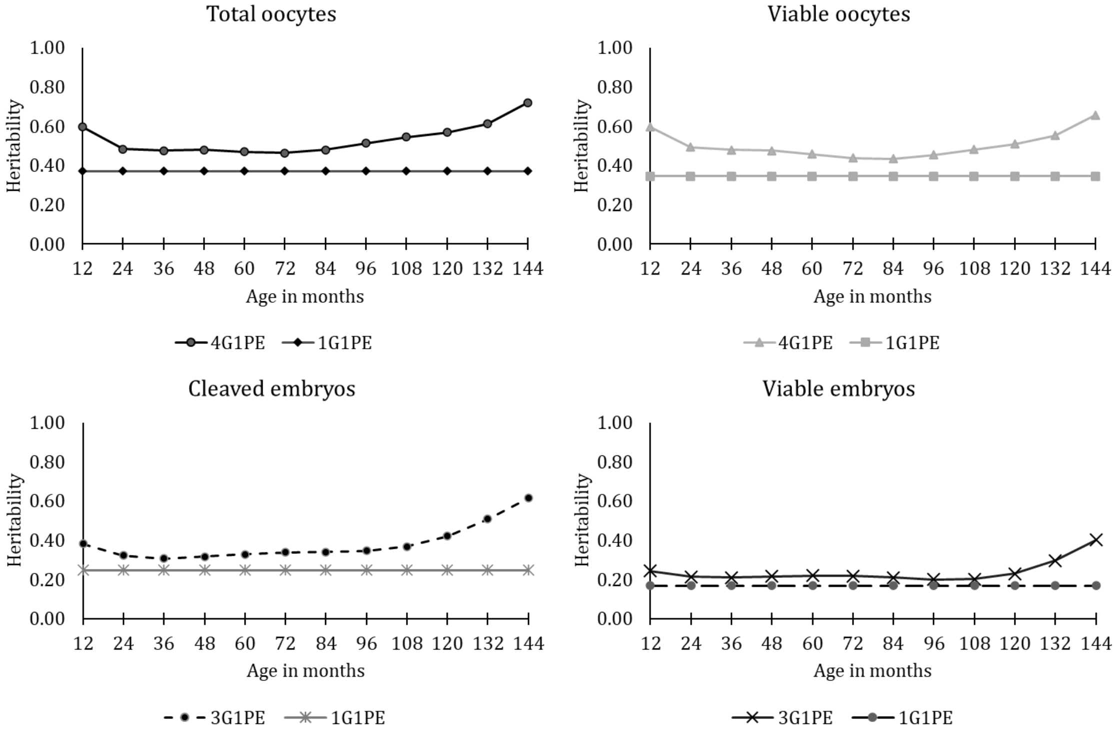

Figure 2 - Heritability estimates for the in vitro embryo production traits using random regression model and repeatability models in Brazilian dairy Gir cattle.

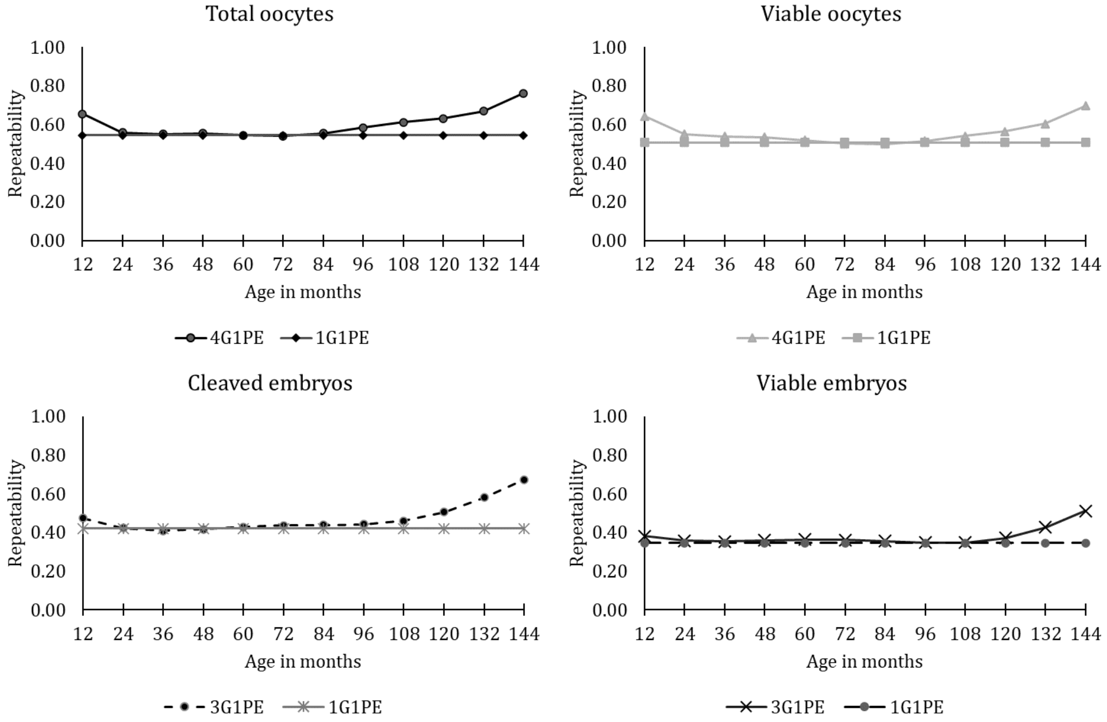

Figure 3 - Repeatability estimates for the in vitro embryo production traits in Brazilian dairy Gir cattle.

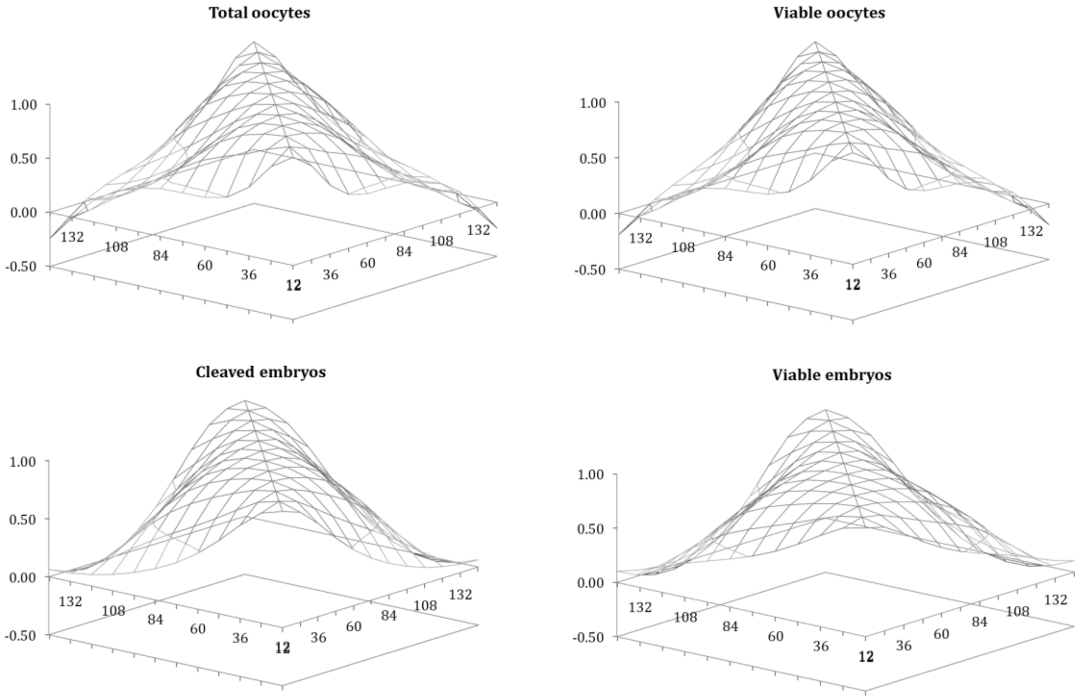

Figure 4 - Genetic correlation estimates between the different collection months for total oocytes (4G1P), viable oocytes (4G1P), cleaved embryos (3G1P), and viable embryos (3G1P) using random regression models in Brazilian dairy Gir cattle.

Table 4 - Spearman's rank correlation between estimated breeding values for total oocytes obtained by 1G1P and 4G1P models at different ages at ovum pick-up, considering the best 5% sires (above diagonal) and 5% females (below diagonal)

Model	1G1P	4G1P_24	4G1P_48	4G1P_72	4G1P_96	4G1P_120	4G1P_144
1G1P		0.71	0.49	0.66	-0.17	0.08	0.02
4G1P_24	0.67		0.58	0.58	0.02	0.86	0.2
4G1P_48	0.76	0.76		0.78	0.27	0.40	-0.02
4G1P_72	0.65	0.56	0.72		0.50	0.22	0.15
4G1P_96	0.01	-0.17	-0.01	0.33		0.74	0.44
4G1P_120	-0.13	-0.42	-0.25	0.06	0.67		0.53
4G1P_144	0.12	-0.08	0.11	0.12	0.57	0.69	

Table 5 - Spearman's rank correlation between estimated breeding values for viable oocytes obtained by models 1G1P and 4G1P at different ages at ovum pick-up, considering the best 5% sires (above diagonal) and 5% females (below diagonal)

Model	1G1P	4G1P_24	4G1P_48	4G1P_72	4G1P_96	4G1P_120	4G1P_144
1G1P		0.79	0.49	0.67	0.50	0.25	0.31
4G1P_24	0.69		0.61	0.58	0.3	0.16	0.58
4G1P_48	0.80	0.74		0.71	0.28	-0.18	0.34
4G1P_72	0.68	0.57	0.76		0.54	0.09	0.43
4G1P_96	0.09	0.06	0.18	0.38		0.63	0.61
4G1P_120	-0.14	-0.25	0.04	0.25	0.62		0.73
4G1P_144	-0.12	0.19	0.33	0.25	0.48	0.68	

Table 6 - Spearman's rank correlation between estimated breeding values for cleaved embryos obtained by 1G1P and 3G1P models at different ages at ovum pick-up, considering the best 5% sires (above diagonal) and 5% females (below diagonal)

Model	1G1P	3G1P_24	3G1P_48	3G1P_72	3G1P_96	3G1P_120	3G1P_144
1G1P		0.22	0.48	0.52	0.59	0.60	0.57
3G1P_24	0.68		0.59	0.40	-0.25	0.19	0.5
3G1P_48	0.75	0.72		0.63	0.34	0.34	0.54
3G1P_72	0.60	0.45	0.78		0.69	0.44	0.19
3G1P_96	0.30	0.18	0.38	0.63		0.69	0.40
3G1P_120	0.05	−0.13	0.01	0.14	0.55		0.69
3G1P_144	−0.16	0.00	0.09	0.21	0.47	0.67	

Table 7 - Spearman's rank correlation between estimated breeding values for viable embryos obtained by 1G1P and 3G1P models at different ages at ovum pick-up, considering the best 5% sires (above diagonal) and 5% females (below diagonal)

Model	1G1P	3G1P_24	3G1P_48	3G1P_72	3G1P_96	3G1P_120	3G1P_144
1G1P		0.73	0.68	0.73	0.74	0.59	0.38
3G1P_24	0.65		0.73	0.55	0.38	0.52	0.84
3G1P_48	0.79	0.79		0.85	0.51	0.43	0.40
3G1P_72	0.77	0.56	0.84		0.77	0.61	0.61
3G1P_96	0.72	0.32	0.56	0.75		0.62	0.35
3G1P_120	0.27	-0.13	0.16	0.21	0.42		0.53
3G1P_144	0.30	0.30	0.17	0.06	0.18	0.60	

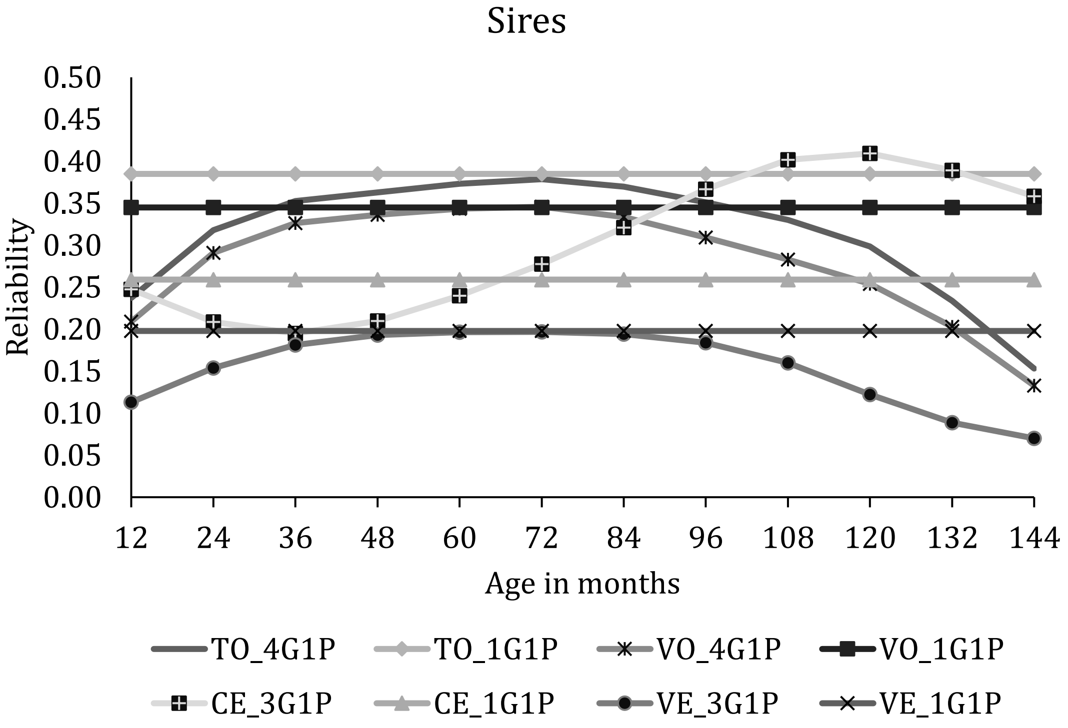

Figure 5 - Reliability of estimated breeding value of 5% best sires for total oocytes (TO), viable oocytes (VO), cleaved embryos (CE), and viable embryos (VE) in Brazilian dairy Gir cattle.

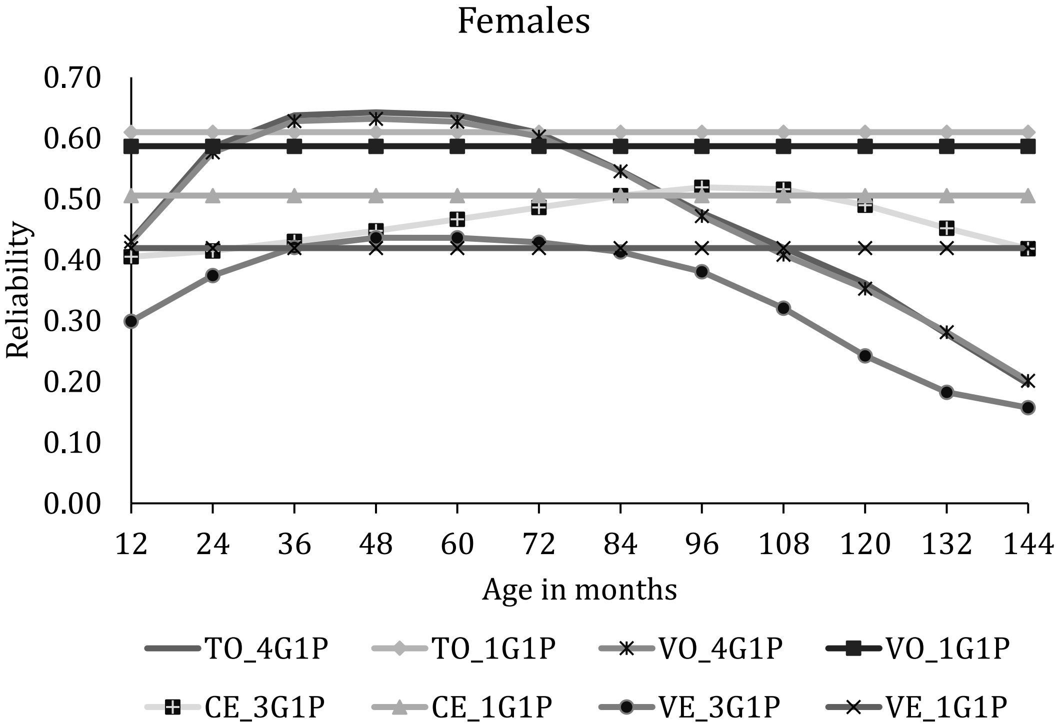

Figure 6 - Reliability of estimated breeding value of 5% best females for total oocytes (TO), viable oocytes (VO), cleaved embryos (CE), and viable embryos (VE) in Brazilian dairy Gir cattle.

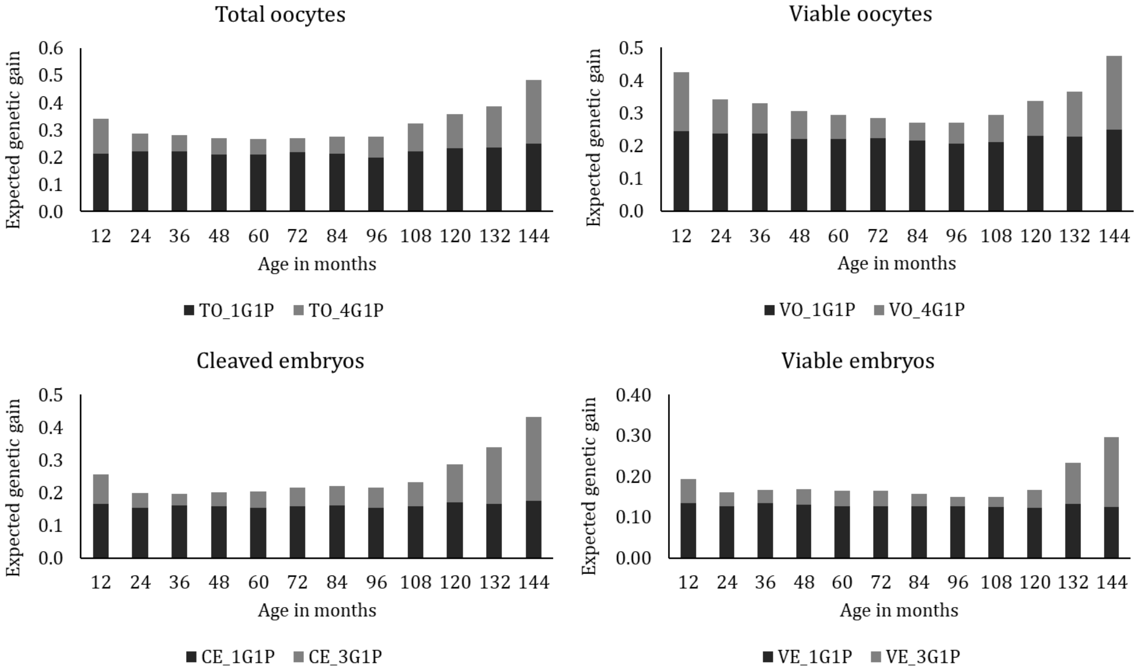

Figure 7 - Expected genetic gain for total oocytes, viable oocytes, cleaved embryos, and viable embryos in Brazilian dairy Gir cattle.

CAPÍTULO III

Impact of heat stress on genetic evaluation of oocytes and embryos production in Gir dairy cattle[4]

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Impact of heat stress on genetic evaluation of oocytes and embryos production in Gir dairy cattle

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ABSTRACT

Identifying and selecting genotypes tolerant to heat stress might improve reproductive traits in dairy cattle, including oocytes and embryos production. The temperature-humidity index (THI) was used via random regression models, to investigate the impact of heat stress on genetic parameters and breeding values of oocytes and embryo production of Gir dairy cattle. We evaluate records of total oocytes (TO), viable oocytes (VO), cleaved embryos (CE), and viable embryos (VE) from donors Gir dairy. Twenty-four models were tested, considering the ages at ovum pick-up (AOPU) and THI means as a regressor in the genetic evaluation. We computed THI in eight periods, from 0 to 112 days before ovum pick-up which were adjusted by different orders of Legendre polynomials (second, third and fourth). The model of best fit according to Akaike’s information criterion (AIC) and Model Posterior Probabilities (MPP) considered Legendre polynomials of third order and THI means of 112 days for TO, fourth-order and 56 days for VO second order and 28 days for CE, and second-order and 42 days for VE, respectively. The heritability (h^2) estimates across AOPU and THI scales range from 0.34 to 0.62 for TO, 0.31 to 0.58 for VO, 0.26 to 0.39 for CE, 0.15 to 0.26 for VE, respectively. The fraction of the phenotypic variance explained by the permanent environment in different AOPU and THI scales range from 0.03 to 0.25 for TO, 0.05 to 0.26 for VO, 0.09 to 0.36 for CE, and 0.15 to 0.27 for VE, respectively. The Spearman rank correlation between the estimated breeding values (EBV) in different AOPU and THI scale from the top 5% sires and females range from: 0.18 to 0.90 for TO, 0.31 to 0.95 for VO, 0.14 to 0.85 for CE, and 0.47 to 0.94 for VE, respectively. The heritability estimates for all evaluated traits vary from moderate to high magnitude across AOPU and THI scales, indicating that genetic selection can result in rapid genetic progress for the evaluated traits. There was a reordering among the best animals in different AOPU and THI. It is possible to select animals tolerant to heat stress to improve oocytes and embryo production in Gir dairy cattle.

Keywords: Temperature-humidity index, *In vitro* fertilization, Random regression models, Heritability, Estimated breeding values

1. Introduction

Heat stress can be defined as a condition that occurs when an animal cannot dissipate an adequate quantity of heat, whether it is produced or absorbed by the body, to maintain body thermal balance (Bernabucci et al., 2014).

Therefore, heat stress negatively impacts cattle welfare and productivity (Lees et al., 2019), and reproductive function in dairy cows (Khan et al., 2020). Moreover, heat stress causes subfertility in cattle by inducing alterations in steroidogenic capacity, follicular function, and ovulation defects, which eventually negatively affect oocyte quality and embryo survival (Stamperna et al., 2020). Perturbations in the physiology of the follicle-enclosed oocyte during the lengthy period of follicular development can potentially lead to an oocyte with reduced competence for fertilization and subsequent development, although the stage of follicular development that is susceptible to thermal stress has not been precisely defined (Gendelman and Roth, 2012). Therefore bovine oocytes production and quality are also susceptible to thermal stress at various stages of follicular development (Gendelman and Roth, 2012).

The identification and selection of genotypes resistant to heat stress for milk production (Bernabucci et al., 2014; Negri et al., 2021a), somatic cell score (Negri et al., 2021b), fat and protein yield (Negri and Cobuci, 2021) are available on the literature. However, to the best of our knowledge, it is the first study to report the impact of heat stress on genetic parameters of oocytes and embryos production on dairy cattle.

The artificial reproductive traits are of great interest to researchers, technicians, and farmers because the use of *in vitro* reproductive biotechnologies in dairy herds is growing fast, and it can result in increased genetic gain by propagating offspring from animals with high genetic merit (De Vries and Kaniyamattam, 2020). The objective of this study was to investigate the impact of heat stress, via THI, in the genetic evaluation for oocytes and embryo production of dairy Gir cattle. Therefore, we evaluated the contribution of THI computed in different periods before ovum pick-up and adjusted by different orders of Legendre polynomials, we demonstrated the impact of THI and AOPU on the ranking and selection of candidates. Additionally, we aimed to quantify the magnitude of genetic variability for heat stress for these traits to implement selection for animals tolerant to heat stress.

2. Material and methods

Data on total oocytes (TO), viable oocytes (VO), cleaved embryos (CE), and viable embryos (VE) were collected from 2008 to 2017 from three herds of Dairy Gir cattle, belonging to the same company, located at the State of Minas Gerais (MG) – Brazil (19º55’ S – 43º57’ W), were evaluated in this study.

The females age at ovum pick-up (AOPU) range from 14 to 145 months (Figure 1). Only healthy females with at least two individual records of ovum pick-up during the age period were maintained. Moreover, semen from 176 different sires (Gir or Holstein) was used in *in vitro* fertilization procedures.

The contemporary groups are defined by the concatenation of herd, year, ovum pick-up, and season. Seasons were classified into wet (October until March) or dry (April until September). Contemporary groups that contained less than five observations were excluded. For the traits, CE, and VE, embryos from sires that were used only once in the *in vitro* fertilization process were excluded.

Therefore, we evaluated 11,343 records of TO and VO from 1,740 donors, and 11,305 records of CE and VE from 1,734 Gir dairy donors. The pedigree file included 5,908 animals.

The statistical program R (R Core Team, 2017) was used for data editing and descriptive statistics (Table 1). The oocyte and embryo counts were transformed using the common logarithmic transformation, $y_i = log(y+1.001)$ where y_i is the log transformed value of oocyte and embryo count and y e the original value of oocyte and embryo count.

Temperature and humidity data were obtained from three weather stations located in Muriaé - MG, Coronel Pacheco - MG, and Viçosa - MG. Data were recorded hourly, resulting in 24 measurements per day from 2007 to 2017. The data from the weather station located in Muriaé – MG was preferable used (94.5%) and the lack of information was filled by information from the other two cited stations. Muriaé is classified as weather aw (tropical wet and dry climate) according to the Köppen-Geiger climate classification, this municipality contains one of the herds analyzed and is 44 and 62 kilometers from the municipalities where the other two herds are.

THI was evaluated according to equation described by National Research Council (NRC, 1971): $THI = \left[(1.8 \times DBT + 32) - \left(0.55 - (0.0055 \times RH) \times (1.8 \times DBT - 26)\right)\right]$ in which DBT is dry bulb temperature in degree Celsius (°C) and RH is relative humidity (%).

After having the THI calculated for seven periods: 7, 14, 21, 28, 42, 56, and 112 days before ovum pick-up, and the average THI for each of these periods (Table 2) was used as a regressor in the random regression models for genetic evaluation (Figure 2). These periods were determined to verify which period of heat stress interferes with the production of oocytes and embryos.

The estimates of variance components for ovum pick-up traits at age (months) and THI were obtained using a random regression model:

$$Y_{ijklm} = \ hys_i + nop_j + \ \sum_{k=0}^{n} \emptyset_k(mo_t)\beta_{jk} + \sum_{k=0}^{n} \emptyset_k(mo_t)u_{jk} +$$

$$\sum_{k=0}^{n} \emptyset_k(mo_t)\ pe_{jk} + \sum_{k=0}^{n} \emptyset_k(thi_l)u_{jk} + \ \sum_{k=0}^{n} \emptyset_k(thi_l)\ pe_{jk} + sa_m + \ e_{ijklm},$$

in which Y_{ijkl} is the vector of trait value (TO, VO, CE, and VE) in month *t* within the herd-year-season of ovum pick-up; hys_i is the fixed effect of contemporary groups of ovum pick-up; β_{jk} is the fixed regression coefficient of the interval between ovum pick-up (IOPU) in the same animal, defined in classes (1 = 0 first ovum pick-up; 2 = 1 to 6 days between ovum pick-up, 3 = 7 to 14 days, 4 = 15 to 21 days, 5 = 21 to 30 days and 6 = more 30 days between ovum pick-up); nop_j is the covariable effect number ovum pick-up in the same animal; sa_m fixed effect referring to sire used in artificial fertilization (effect used only for CE and VE); u_{jk} and pe_{jk} are random regression coefficients that describe, respectively, the additive genetic and permanent environment effects on the performance of animal j; Ø$_{\text{k}}$(mo_t) is Legendre polynomial for ovum pick-up of animal *j* in month *t*, Ø$_{\text{k}}$(thi_l) is Legendre polynomial for ovum pick-up of animal *j* in THI *l*, in which *k* is Legendre polynomial coefficient; and e_{ijklm} is the random error.

The (co)variance structure was as follows:

$$Var\begin{bmatrix} \boldsymbol{u} \\ \boldsymbol{pe} \\ \boldsymbol{e} \end{bmatrix} = \begin{bmatrix} \mathbf{A} \otimes \mathbf{G} & 0 & 0 \\ 0 & \boldsymbol{I} \otimes \boldsymbol{P} & 0 \\ 0 & 0 & \boldsymbol{E} \end{bmatrix},$$

in which ***G*** and ***P*** are the covariance matrices of the random regression coefficients, ***A*** is the kinship matrix, ***I*** is the identity matrix, ***E*** = $\sigma_{\boldsymbol{e}}^2$ is a diagonal (residual) matrix, and ⊗ is Kronecker product between the matrices.

To assess which model had the best fit to be used in the genetic evaluation of traits of artificial reproduction, we sought to adjust the fixed curve (IOPU), genetic and permanent environment effects using different polynomial functions from the two (M2), three (M3), and four order (M4), and average THI for different periods before ovum pick-up (0, 7, 14, 21, 28, 42, 56, and 112 days(D)), totaling 24 different combinations between genetic additive and permanent environmental effects for AOPU and THI. For instance, the model SM2 does not consider THI and considered Legendre polynomial of order 2 for AOPU while the model D7M3 considered THI computed at 7 days before ovum pick up and Legendre polynomial of order 3 for AOPU (Table 3).

The estimated breeding value (EBV) of an animal *i* was computed using AOPU and THI information, according to the equation, $EBV_i^{l,t} = \varphi_{(l)t}\hat{a}_i'$, where $\hat{a}_i'$ is the vector of the estimated additive genetic values for the orthogonal regression coefficients of animal *i* (coefficients corresponding to AOPU and THI) and $\varphi_{(l)t}$ is a vector of the orthogonal coefficients evaluated in THI *l* and AOPU *t*.

All analyzes were estimated through the maximum restricted likelihood method, using the program REMLF90 (Misztal et al., 2002).

The goodness-of-fit was carried by Akaike's information criterion ($AIC = -2\log L$+2*p*, in which *p* is the number of parameters in the model). To complement this information, the Model Posterior Probabilities (MPP) were calculated, as presented by Wilberg and Bence (2008), which is given by: $p(M_n|\theta) = \exp\left(-\frac{\Delta_n}{2}\right) / \sum_{n=1}^{24}\exp\left(-\frac{\Delta_n}{2}\right)$, *n*: range from 1 = SM2 to 24 = D112M4 in which $p(M_t|\theta)$ is the posteriori probability of model *n* to be the best among the set of models compared, Δ_n is the AIC difference between model *n* and the model that presented the smallest AIC value. The best model was used to make inferences about the parameters of interest. The phenotypic means of the traits considering the best models are available in Figure 3.

To verify the occurrence of changes in the ranking of the best animals at different THI, we calculated the Spearman rank correlation between estimated breeding value, for each trait across selected models, of the top 5% sires or females.

The top 5% sires and 5% females selected by EBV in 20 months at THI equal 80 (EBV20_80), were sampled to represent the EBV of the trait (TO, VO, CE, and VE) around the AOPU, and the resilience via EBV HS (heat stress) of the animals. The EBV20_80 was selected because is younger age, which is important for the reduction of the generation interval, and THI 80 because it is close to the mean and median of the database used.

3. Results

According to AIC and MPP, the best goodness of fit model for each trait was D112M3 for TO, D56M4 for VO, D28M2 for CE, D42M2 for VE (Table 4). Therefore, Legendre polynomial of third order for AOPU and period of 112 days for THI should be used to estimate genetic parameters for TO; Legendre polynomial of fourth order for AOPU and period of 56 days for THI should be used to estimate genetic parameters for VO; while Legendre polynomial of second order for AOPU and period of 28 days for THI should be used to estimate genetic parameters for CE. However, we observed a small difference between the MPP of models D42M2 (0.25) and D14M2 (0.24) for VE, indicating that Legendre polynomial of second order for AOPU and either period of 42 or 14 days for THI can be used to estimate genetic parameters for VE.

The heritability estimates by the best goodness of fit model for each trait range from 0.34 to 0.62 for TO (Figure 4A), 0.31 to 0.58 for VO (Figure 4B), 0.26 to 0.39 for CE (Figure 4C), and 0.15 to 0.26, for VE (Figure 4D), respectively. The high heritability estimates of 0.62 for TO and 0.58 for VO were reported at the extremes of AOPU and THI scales. Heritability estimates for CE showed small variation across the THI scale and the higher heritability estimates were observed at younger AOPU. The heritability estimates for VE were high in larger THI and at the extremes (beginning and ending) of the AOPU.

The fraction of the phenotypic variance explained by the permanent environment (c^2) range from small to moderate magnitude and the highest values were reported in the greater AOPU and THI. The c^2 range from 0.03 to 0.25 for TO, (Figure 5A), from 0.05 to 0.26 for VO, (Figure 5B) from 0.09 to 0.36 for CE (Figure 5C), and from 0.15 to 0.27 for VE (Figure 5D), respectively.

Spearman rank correlation for the EBV of top 5% sires and top 5% females for each trait evaluated at 20, 60, 100, and 140 months of AOPU and at 70, 75, 80, and 85 THI are demonstrated in Figures 6A (TO), 6B (VO), 6C (CE), and 6D (VE). Considering the same age and different THI selection. High Spearman rank correlations of 0.90 to 0.99 for TO, 0.69 to 0.99 for VO, 0.99 TO 1 for CE, 0.98 to 1 for VE, respectively were reported at the constant AOPU and varying THI. For instance, for TO at 20 months AOPU and THI = 70 we observed Spearman correlation between for EBV of top 5% sires of 0.97 for THI = 75; 0.97 for THI =

80 and 0.96 for THI = 85, and for 5% females of 0.98 for THI = 75; 0.97 for THI = 80 and 0.94 for THI = 85, respectively.

However, at constant THI and varying AOPU, the Spearman correlation between the EBV of top 5% sires and females for each trait evaluated vary from 0.18 to 0.89 for TO, 0.41 to 0.95 for VO, 0.16 to 0.85 for CE, 0.45 to 0.92 for VE, respectively indicating significant ranking reordering of candidates. For instance, for TO at 20 months AOPU and THI = 70 we observed Spearman correlation between EBV of top 5% sires of 0.76 for 60 months of AOPU; 0.53 for 100 months of AOPU, and 0.25 for 140 months of AOPU. While the Spearman correlation between EBV of top 5% of females were 0.87 for 60 months of AOPU; 0.74 for 100 months of AOPU and 0.59 for 140 months of AOPU, respectively. We also observed a large variation of Spearman correlation between EBV of top 5% sires and 5% top females at different AOPU of 0.18 to 0.90 for TO, 0.31 to 0.95 for VO, 0.14 to 0.85 for CE, 0.47 to 0.94 for VE, respectively.

The genetic breeding value of the top 5% sires and females selected by EBV at 20 months and THI equal 80 showed substantial changes across AOPU scale for TO (Figure 7a, 7b), VO (Figure 7c, 7d), CE (Figure 7e, 7f) and VE (Figure 7g, 7h). Specially at the extremes AOPU values (20 and 140 Months), showing that animals with high EBV at young ages (20 Months) had low EBV at advanced ages (140 months) and vice versa, on the other hand, small variation were observed for EBV of the top 5% sires and females selected by EBV at 20 months and THI equal to 80 across the THI scale for TO (Figure 8a, 8b), CE (Figure 8e, 8f) and VE (Figure 8g, 8h). While higher EBV HS was reported at the extremes of the THI scale for VO (Figure 8c, 8d), being possible in this way, select the animals that left offspring that will produce higher oocyte and embryo counts, even at high THI.

In the case of CE (Figure 8e, 8f) the EBV HS changed little between the different THI (70 to 85), and in the case of VE (Figure 8g, 8f), the EBV HS had an increasing linear behavior as the THI value increased. The EBV HS (Figure 8) varied considerably (on a logarithmic scale) among of the top 5% sires and top 5% females selected by EBV (20 months at THI equal to 80), ranging from 0.33 to -0.05 for TO, 0.18 to -0.08 for VO, 0.26 to -0.005 for CE, 0.23 to 0.01 for VE,

4. Discussion

The period in which heat stress can affect oocyte and embryo production is not yet clearly defined, in this work, different periods were the best for each trait, longer periods (112 and 56 days) were better for TO and VO, and shorter periods (28 and 42 days) were the best for CE and VE, respectively. Torres-Júnior et al. (2008) showed that the negative effects of heat stress can persist for 105 days after the exposure period in Gir dairy cattle, Al-Katanani et al. (2002) show that even after a 42-day cooling period, oocytes from Holstein cows exposed to heat stress had reduced quality, demonstrating heat stress has an effect that persists for a long time, even after the animals are in thermal comfort.

The follicular development takes approximately 180 days, and it is well known that the primordial, primary, and secondary follicles are heat resistant, but no clear data to support the heat sensitivity of primordial follicles while developing antral follicles, including dominant and preovulatory follicles (Roth, 2017). In the current study, we identified the best goodness of fit models with specific THI periods for each trait, suggesting that heat stress might impact the oocytes and embryo production. Moreover, Torres-Júnior et al. (2008) reported a negative impact of heat stress on follicular growth and oocyte function even before the antral phase (42 days) or primary follicle (85 days) reducing the potential of oocyte development for a period longer than two or three estrous cycles in Gir cows.

The heat stress, THI higher than 69, resulted in milk yield losses for dairy Gir animals (Santana et al., 2015) and it is important to note that all THI values computed in this study (Table 2) were higher than the threshold of THI 69 (see table 1) suggested by Santana et al. (2015). For instance, in our study, 97.4% of data collected for TO, 96.7% for CE, and 96.6% for VE were collected with THI higher than 69.

The best goodness of fit models considered Legendre polynomial of order two for CE and VE, and order 3 for TO. Similarly, Negri et al. (2021a) reported that the best model to evaluate heat stress for milk yield in Holstein cattle raised in Minas Gerais-Brazil uses order two of Legendre polynomials and Santana et al. (2015) also indicated order two of Legendre polynomials for the genetic effect of THI to evaluate the detrimental effects of continuous selection for milk yield in Dairy Gir cattle. It is important because the selection model should balance the goodness of fit and its complexity or the number of parameters estimated (Li et al., 2020).

A model with Legendre polynomial of fourth-order (D56M4) was indicated as the most suitable for VO and it presented higher MPP when compared with other models evaluated here (0.99 versus 0), showing that the chosen model was well suited to the data. The model choice is crucial for the quality and accuracy of the genetic evaluation because a model with better goodness of fit does not necessarily indicate better predictive capacity, however, a model with an inadequate fit, such as underfit, induces bias (Li et al., 2020).

The heritability estimates here were higher than the values previously reported in the literature, for instance, 0.31 for TO for Holstein cattle raised in the United States (Cornelissen et al., 2017), 0.32 for VO for Brazilian Dairy Gir cattle (Vizoná et al., 2020), 0.17 for CE for Holstein cattle raised in Canada (Jaton et al., 2016), and 0.14 for VE for Guzerá cattle raised in Brazil (Perez et al., 2016).

The high heritability estimates reported here may be partially explained by the similarity on animal husbandry techniques applied in the three farms evaluate, which standardize management, which contributes to the reduction of environmental variation and highlights genetic differences among animals.

Peixoto et al. (2004) reported that the highest estimate of heritability for a number of viable embryos when only data from the first flush were considered, compared to data from the first three flushes, as an explanation for this difference, is that the handlings in the first flush, were standardized for all the donors, however in the following flushes, the handlings and mainly the hormonal doses were adjusted according to the previous response of the donor, and this practice could result in underestimated heritability values.

Another explanation for the high heritability estimates reported in our study is that these traits do not undergo any direct genetic selection, which avoids the Bulmer effect on these traits.

Variations in heritability values were verified along the AOPU and THI scale, however, changes in the heritability values of TO, VO, and CE along the

THI scale were more modest than in the AOPU trajectory. Santana et al. (2015) reported small changes in genetic, permanent environment, and residual variances for milk yield on the test day in dairy Gir on the THI scale. Changes in heritability estimates for test-day milk yield in Holstein cattle, on the THI scale, were reported by Negri et al. (2021a), with lower heritability reported from the heat stress threshold for that population (THI>74). According to Brügemann et al. (2011), heat stress can suppress the expression of the genetic potential of animals, however, in our study we found high heritability estimates at high THI, indicating the existence of considerable additive genetic variation that can be exploited by selection. This is particularly important in tropical climates, where high THI values can be achieved, particularly in summer. Habeeb et al. (2022) highlights that high THI in the spring and summer season affects physiological body functions, affecting the feed intake and feed efficiency, blood biochemistry components, and blood hormones. And even under these conditions, there is genetic variability that allows the selection of animals adapted to these conditions in the dairy Gir herd evaluated.

A considerable variation in c^2 values across the AOPU and THI scale was reported mainly for CE, TO and VO and less variation was reported in the estimation of c^2 for VE. The fraction of the phenotypic variance explained by the permanent environment is not usually presented in scientific works, however, as it is easily calculated by the difference between repeatability and heritability. Thus it can be verified in the Brazilian Guzerá breed (Perez et al., 2017b) that the effect of permanent environment is greater than the effect genetic for traits VO, CE, and VE.

In our study, the values of heritability and c^2 were similar for CE and VE, while the heritability values were higher than c^2 for TO and VO. However, less effect of permanent environment compared to genetic for traits VO and VE, with c^2 values similar to those reported in our study by the repeatability model where estimated for Holstein cattle raised in the Netherlands (Cornelissen et al., 2017). These differences pointed out between the studies, are due to the environments where these animals were raised. Thus, additive genetic effect influences future production more than the permanent environmental effect for TO and VO, in the population of this study.

The fraction of the phenotypic variance explained by the permanent environment can be used when selecting animals right after the first ovum pick-up, a high c^2 value can be associated with high repeatability, and this facilitates the selection of young donors for the next ovum pick-up.

The genotype by environment interactions due to heat stress can be identified by the reordering in the ranking of bulls across extreme THI values (Santana et al., 2015). We observed ranking reordering of the best animals when evaluated at different AOPU and THI for all traits evaluated here, indicating that there was genotype by environment interaction for oocytes and embryo production.

The heat stress impact on embryo production was more evident in *Bos taurus* than in *Bos indicus* (Paula-Lopes et al., 2013). For instance, the difference between the production of total oocytes, viable oocytes, and embryos counts evaluated on the hot and cold seasons for *Bos taurus* was smaller than the values reported for *Bos indicus* (Fernandes et al., 2014). For example, the average number of embryos in the hot season was 1.2 and 4.9 for *Bos taurus* and *Bos indicu*s, respectively. Similarly, the number of embryos in the cold season was 2.9 and 4.6 for *Bos taurus* and *Bos indicu*s, respectively (Fernandes et al., 2014).

Moreover, losses in milk production per THI unit were relatively small in dairy Gir cattle than the Holstein breed (Santana et al., 2015), confirming that *Bos indicu*s might be more resilient to heat stress than the *Bos taurus*. The impact of heat stress on the production trait of the Holstein breed is well explored in the literature (Roth, 2017). For instance, studies in the United States (Bohmanova et al., 2007), Italy (Bernabucci et al., 2014), and Brazil (Negri et al., 2021a) confirmed the effect of heat stress on milk yield.

The low estimates of Spearman correlations between EBV of top individuals evaluated in different AOPU and THI for all traits suggested reranking of animals. Therefore, the oocyte quality can vary under heat stress with a negative impact on blastocyst yield and it can partially explain the limited success rate of *in vitro* fertilization (Lonergan and Fair, 2016).

Dairy cows in moderate climates might be more affected by heat stress than cows acclimatized to tropical or subtropical climates, which are exposed to constant heat stress (Schüller et al., 2014), as is the case of the dairy Gir that is the object of research in this study.

The dairy Gir population is heading toward a higher milk yield level at the expense of lower tolerance to heat stress due the genetic antagonism between production and tolerance to heat stress, demonstrated by the negative genetic correlation between these components (Santana et al., 2015). The variability of the EBV HS in THI = 85, of the top 5% sires and top 5% females selected for EBV at 20 months and THI equal 80, for the TO trait was 0.24 to -0.01 in sires, and 0.33 to -0.02 in females, for VO it was from 0.14 to 0.03 in sires, and 0.18 to 0.01, for CE range was 0.19 to -0.005 in sires, and 0.26 to -0.006 in females, and for VE range was 0.23 to 0.02 in sires, and 0.24 to 0.02 in females, thus demonstrating a considerable variation between the EBV HS of the animals, generating the possibility of selecting animals tolerant to high THI values. As dairy Gir become more productive, they also become more sensitive to heat stress, as they generate more metabolic heat, among other factors (Bernabucci et al., 2010).

Selection for production would lead to a reduction in the genetic tolerance to heat stress (Ravagnolo et al., 2000), and have led to a decline in fertility (Pryce et al., 2004), in the last 60 years conception rate in high yielding dairy cows decreased from 55% to 35% worldwide (Schüller et al., 2014).

The EVB HS in all traits showed that it is possible to carry out the selection for animals that are more tolerant to heat stress, in addition to EBV in different AOPU, it is possible to select animals with high oocyte and embryo counts early. The earlier the heat stress was detected, the greater the chances of keeping more resilient animals in production and, consequently, the more productive they are in different heat conditions (Negri et al., 2021a).

Combining information from many traits into a single selection index is an important tool for modern cattle breeders because many traits have value to farmers, and single-trait selection can result in undesirable changes in correlated traits (Cole and VanRaden, 2018). A selection index that combines productive and artificial reproduction traits could be useful to breeders when choosing animals submitted to this biotechnology.

However, not all farmers or production systems are interested in artificial reproductive traits, thus it may not be important to include them in an well-established national selection index (Jaton et al., 2016). However, the availability of EBV of these characteristics can be useful to farmers who are interested in producing and commercializing embryos. A high EBV to produce oocytes and/or embryos can be an important selection criterion for dams of sires, since most of the sires with high genetic value for traits of greater economic interest come from embryo transfer. Thus, the creation of a selection index that contemplates these characteristics needs to be evaluated within each breeding and selection objectives.

5. Conclusion

The heritability estimates for oocytes and embryo production varied from moderate to high magnitude across age at ovum pick-up and temperature-humidity index scales, indicating that genetic selection can result in rapid genetic progress.

The heat stress measured by the temperature-humidity index of days before ovum pick-up, is a factor that can impact the selection of animals tolerant to heat stress. In addition, we observed that there is a specific association with heat stress impacts on genetic evaluation. The period of 28 days before ovum pick-up is the most suitable to define the temperature-humidity index used by genetic evaluation models for heat stress in cleaved embryos, on the other hand, longer periods of 42 days are indicated for viable embryos; 56 days for viable oocytes; and 112 days for the characteristic total oocytes. Similarly, order of Legendre polynomials used to adjust the effects of the models is also trait specific, fourth-order Legendre polynomials are indicated for use for viable oocytes; third-order, for total oocytes; and second-order; for cleaved embryos and viable embryos.

The effect of heat stress via the temperature-humidity index should be considered when estimating genetic parameters and predicted breeding values for total and viable oocytes and cleaved and viable embryos production in dairy Gir cattle. Moreover, it should also be considered in the mating selection because the ranking of candidates changed across ages at ovum pick-up and temperature-humidity index. The selection of candidates tolerant to heat stress can greatly help breeders, and as a consequence, increase the reproductive rate of females with greater commercial interest, such as those with high breeding value for economically important traits for different production systems.

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Table 1 - Summary statistics for oocytes and embryo traits (before log transformation) of Dairy Gir cattle

Trait	Mean	Median	SD	Max	Min
TO	21.71	18	15.27	157	1
VO	16.25	13	12.91	142	1
CE	12.01	10	8.85	98	0
VE	4.61	3	4.58	43	0

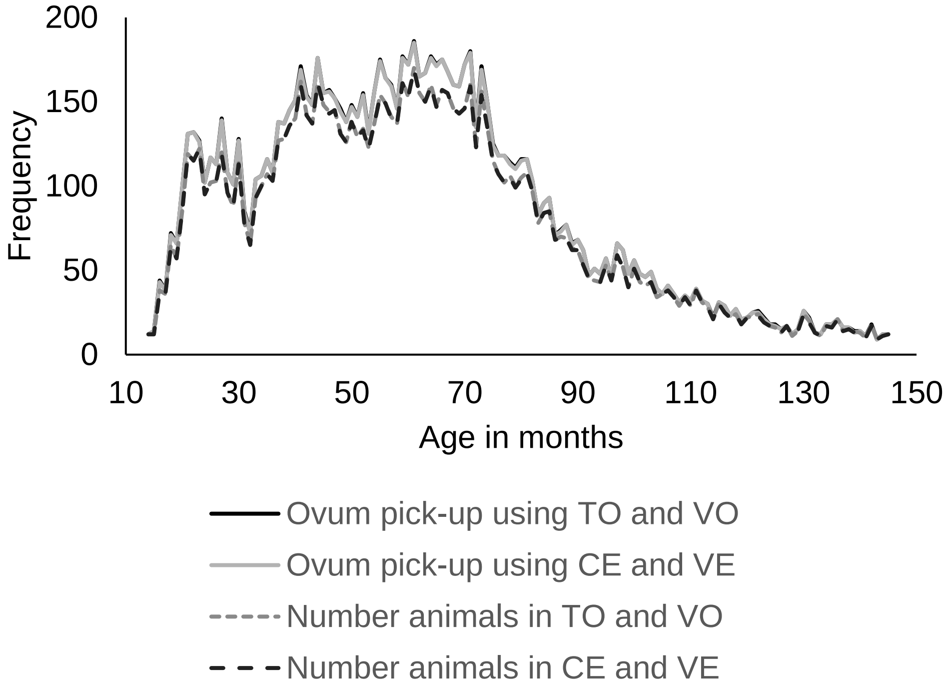


Figure 1 – Frequency of ovum pick-up and number of animals in the age period evaluated for traits total oocytes (TO), viable oocytes (VO), cleaved embryos (CE) and viable embryos (VE).

Table 2 – Summary statistics for THI in different periods.

Period	Mean	Median	SD	Max	Min
D7	78.67	80	5.81	90	62
D14	78.79	79	5.65	89	64
D21	78.65	80	5.53	89	66
D28	78.65	79	5.53	88	67
D42	78.71	79	5.46	88	68
D56	78.72	79	5.41	88	68
D112	78.51	78	4.75	86	70

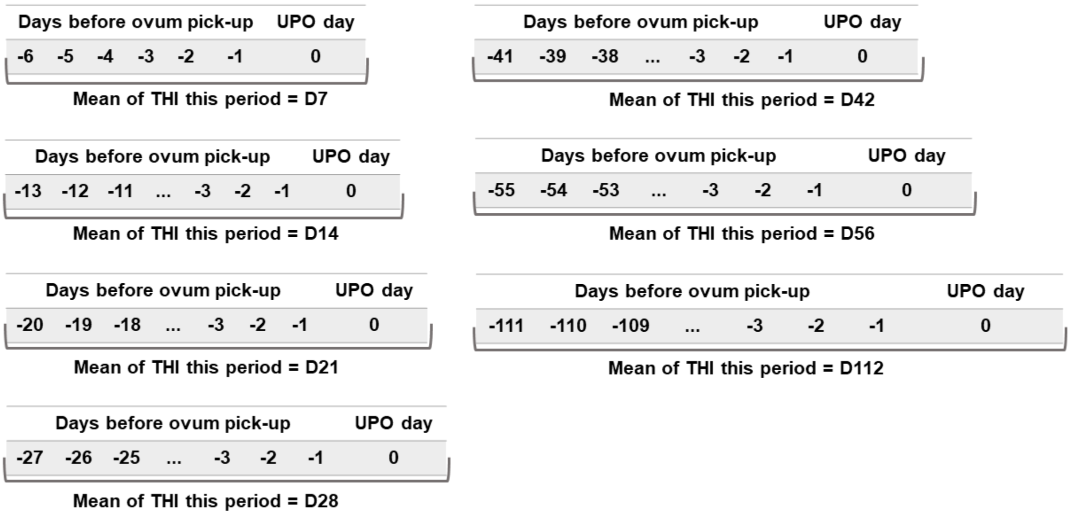


Figure 2 – Schematic representation of the obtaining the average THI of the different periods.

Table 3 – Model's design.

Fixed effects				**Regressor THI**		**Regressor AOPU**		**Model**
CG	IOPO	NOPU	SS[1]	A	P	A	P	
*	++	*	*	-	-	++	++	SM2
*	+++	*	*	-	-	+++	+++	SM3
*	++++	*	*	-	-	++++	++++	SM4
*	++	*	*	++	++	++	++	D7M2
*	+++	*	*	+++	+++	+++	+++	D7M3
*	++++	*	*	++++	++++	++++	++++	D7M4
*	++	*	*	++	++	++	++	D14M2
*	+++	*	*	+++	+++	+++	+++	D14M3
*	++++	*	*	++++	++++	++++	++++	D14M4
*	++	*	*	++	++	++	++	D21M2
*	+++	*	*	+++	+++	+++	+++	D21M3
*	++++	*	*	++++	++++	++++	++++	D21M4
*	++	*	*	++	++	++	++	D28M2
*	+++	*	*	+++	+++	+++	+++	D28M3
*	++++	*	*	++++	++++	++++	++++	D28M4
*	++	*	*	++	++	++	++	D42M2
*	+++	*	*	+++	+++	+++	+++	D42M3
*	++++	*	*	++++	++++	++++	++++	D42M4
*	++	*	*	++	++	++	++	D56M2
*	+++	*	*	+++	+++	+++	+++	D56M3
*	++++	*	*	++++	++++	++++	++++	D56M4
*	++	*	*	++	++	++	++	D112M2
*	+++	*	*	+++	+++	+++	+++	D112M3
*	++++	*	*	++++	++++	++++	++++	D112M4

[1] SS = Service sire, the fixed effect of sire was included only for CE and VE traits.

TO - total oocytes; VO - viable oocytes; CE - cleaved embryos; VE - viable embryos.

CG = Contemporary group (same herd-year-season of ovum pick-up), IOPU = Interval between ovum pick-up, NOPU = Number of ovum pick-up.

A = Additive genetic effect, P = Permanent environment effect.

* Considered; - not considered; + order of the Legendre polynomial (varying from 2 to four order).

Table 4 – Selection criteria based on values Akaike's information criterion (AIC) and Model Posterior Probabilities (MPP) for Total Oocytes (TO), Viable Oocytes (VO), Cleaved Embryos (CE), and Viable Embryos (VE) using random regression models in dairy Gir cattle.

	AIC				MPP			
	TO	VO	CE	VE	TO	VO	CE	VE
SM2	-1871	842	1853	6937	0.00	0.00	0.00	0.01
SM3	-1882	829	1869	6943	0.00	0.00	0.00	0.00
SM4	-1866	833	1911	6987	0.00	0.00	0.00	0.00
D7M2	-1891	823	1845	6934	0.00	0.00	0.00	0.05
D7M3	-1909	800	1868	6947	0.00	0.00	0.00	0.00
D7M4	-1882	819	1920	6996	0.00	0.00	0.00	0.00
D14M2	-1898	814	1842	6931	0.00	0.00	0.01	0.24
D14M3	-1905	797	1864	6945	0.00	0.00	0.00	0.00
D14M4	-1885	811	1910	6989	0.00	0.00	0.00	0.00
D21M2	-1904	810	1837	6932	0.00	0.00	0.13	0.19
D21M3	-1910	791	1859	6946	0.00	0.00	0.00	0.00
D21M4	-1894	804	1885	6981	0.00	0.00	0.00	0.00
D28M2	-1913	804	**1834**	6933	0.00	0.00	**0.45**	0.12
D28M3	-1923	783	1858	6948	0.00	0.00	0.00	0.00
D28M4	-1912	788	1892	6983	0.00	0.00	0.00	0.00
D42M2	-1920	802	1835	**6931**	0.00	0.00	0.25	**0.25**
D42M3	-1929	783	1861	6943	0.05	0.00	0.00	0.00
D42M4	-1913	794	1911	6980	0.00	0.00	0.00	0.00
D56M2	-1922	805	1837	6933	0.00	0.00	0.14	0.10
D56M3	-1928	788	1860	6941	0.03	0.00	0.00	0.00
D56M4	-1933	**772**	1906	6982	0.39	**0.99**	0.00	0.00
D112M2	-1930	806	1842	6936	0.09	0.00	0.01	0.03
D112M3	**-1934**	796	1863	6954	**0.43**	0.00	0.00	0.00
D112M4	-1913	817	1911	6980	0.00	0.00	0.00	0.00

In bold, are the lowest values of AIC and higher values of MPP.

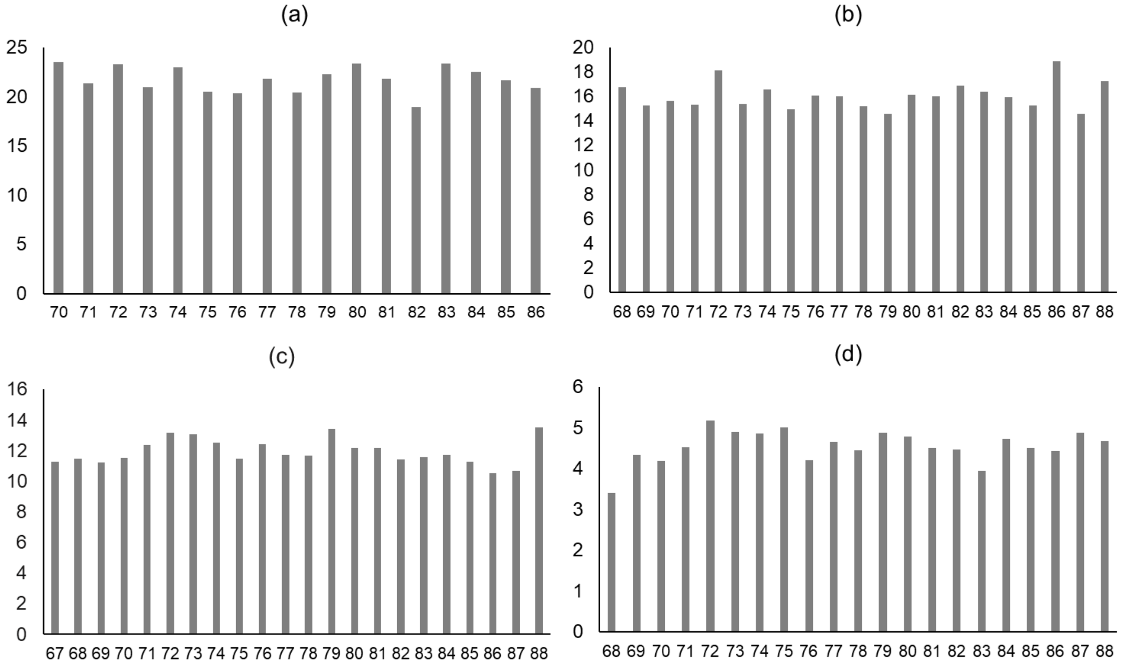


Figure 3 – Phenotypic averages of traits Total Oocytes (a), Viable Oocytes (b), Cleaved Embryos (c), Viable Embryos (d) according to temperature-humidity index (THI) and age at ovum pick-up (AOPU) using the best model for each trait, D112M3 for TO, D56M4 for VO, D28M2 for CE, and D42M2 for VE.

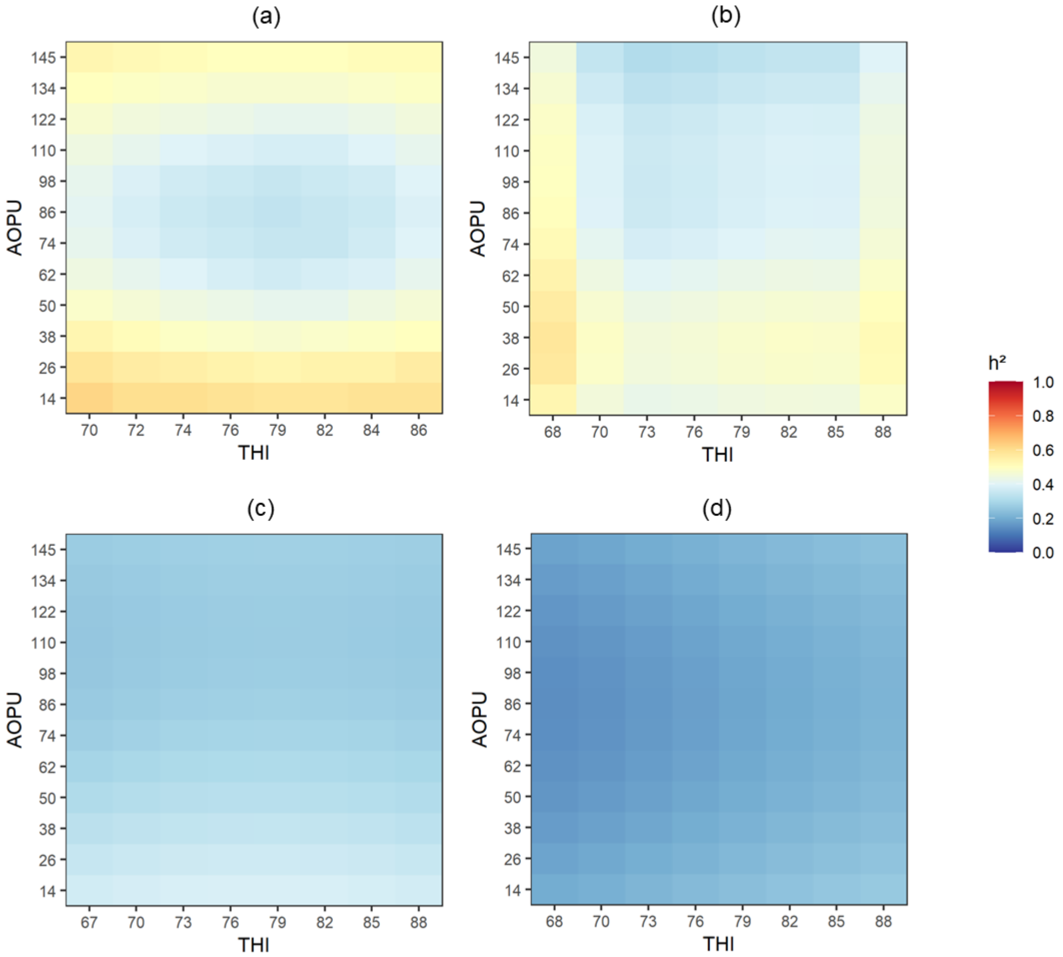


Figure 4– Estimated heritability (h^2) for Total Oocytes (a), Viable Oocytes (b), Cleaved Embryos (c), Viable Embryos (d) according to temperature-humidity index (THI) and age at ovum pick-up (AOPU) using the best model for each trait, D112M3 for TO, D56M4 for VO, D28M2 for CE, and D42M2 for VE.

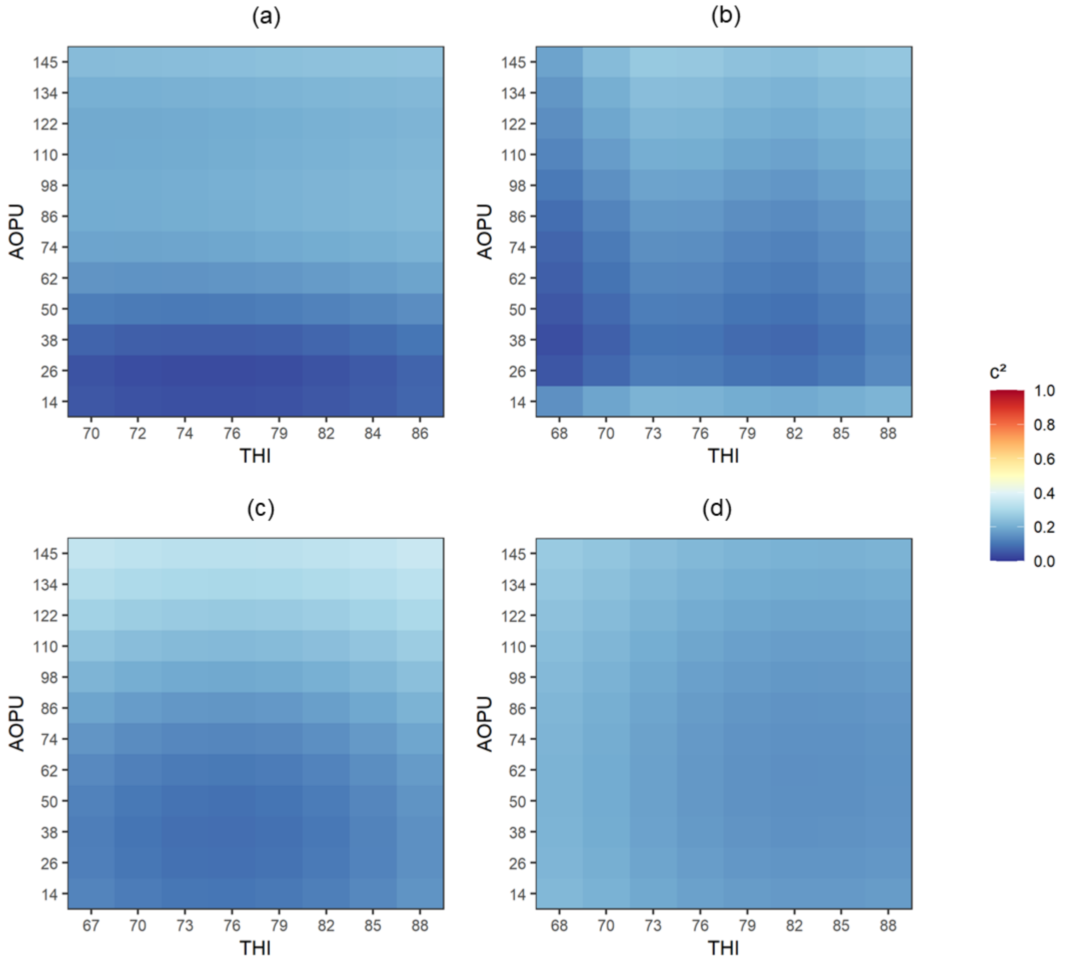
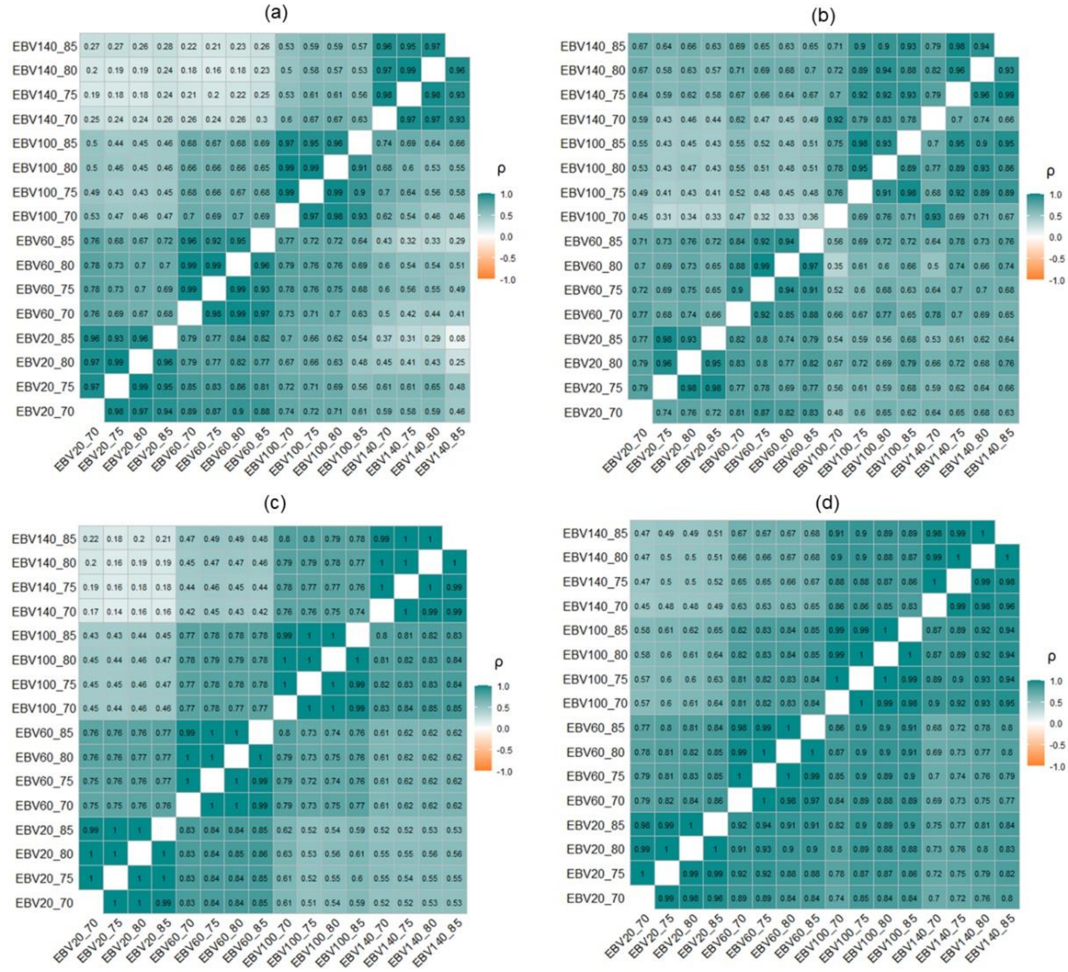


Figure 5 – Estimated fraction of the phenotypic variance explained by the permanent environment (c²) for Total Oocytes (a), Viable Oocytes (b), Cleaved Embryos (c), Viable Embryos (d) according to temperature-humidity index (THI) and age at ovum pick-up (AOPU) using the best model for each trait, D112M3 for TO, D56M4 for VO, D28M2 for CE, and D42M2 for VE.



(a)

	EBV20_70	EBV20_75	EBV20_80	EBV20_85	EBV60_70	EBV60_75	EBV60_80	EBV60_85	EBV100_70	EBV100_75	EBV100_80	EBV100_85	EBV140_70	EBV140_75	EBV140_80	EBV140_85
EBV140_85	0.27	0.27	0.26	0.28	0.22	0.21	0.23	0.26	0.53	0.59	0.59	0.57	0.96	0.95	0.97	
EBV140_80	0.2	0.19	0.19	0.24	0.18	0.16	0.18	0.23	0.5	0.58	0.57	0.53	0.97	0.99		0.96
EBV140_75	0.19	0.18	0.18	0.24	0.21	0.2	0.22	0.25	0.53	0.61	0.61	0.56	0.98		0.98	0.93
EBV140_70	0.25	0.24	0.24	0.26	0.26	0.24	0.26	0.3	0.6	0.67	0.67	0.63		0.97	0.97	0.93
EBV100_85	0.5	0.44	0.45	0.46	0.68	0.67	0.68	0.69	0.97	0.95	0.96		0.74	0.69	0.64	0.66
EBV100_80	0.5	0.46	0.45	0.46	0.66	0.66	0.66	0.65	0.99	0.99		0.91	0.68	0.6	0.53	0.55
EBV100_75	0.49	0.43	0.43	0.45	0.68	0.66	0.67	0.68	0.99		0.99	0.9	0.7	0.64	0.56	0.58
EBV100_70	0.53	0.47	0.46	0.47	0.7	0.69	0.7	0.69		0.97	0.98	0.93	0.62	0.54	0.46	0.46
EBV60_85	0.76	0.68	0.67	0.72	0.96	0.92	0.95		0.77	0.72	0.72	0.64	0.43	0.32	0.33	0.29
EBV60_80	0.78	0.73	0.7	0.7	0.99	0.99		0.96	0.79	0.76	0.76	0.69	0.6	0.54	0.54	0.51
EBV60_75	0.78	0.73	0.7	0.69	0.99		0.99	0.93	0.78	0.76	0.75	0.68	0.6	0.56	0.55	0.49
EBV60_70	0.76	0.69	0.67	0.68		0.98	0.99	0.97	0.73	0.71	0.7	0.63	0.5	0.42	0.44	0.41
EBV20_85	0.96	0.93	0.96		0.79	0.77	0.84	0.82	0.7	0.66	0.62	0.54	0.37	0.31	0.29	0.08
EBV20_80	0.97	0.99		0.96	0.79	0.77	0.82	0.77	0.67	0.66	0.63	0.48	0.45	0.41	0.43	0.25
EBV20_75	0.97		0.99	0.95	0.85	0.83	0.86	0.81	0.72	0.71	0.69	0.56	0.61	0.61	0.65	0.48
EBV20_70		0.98	0.97	0.94	0.89	0.87	0.9	0.88	0.74	0.72	0.71	0.61	0.59	0.58	0.59	0.46

(b)

	EBV20_70	EBV20_75	EBV20_80	EBV20_85	EBV60_70	EBV60_75	EBV60_80	EBV60_85	EBV100_70	EBV100_75	EBV100_80	EBV100_85	EBV140_70	EBV140_75	EBV140_80	EBV140_85
EBV140_85	0.67	0.64	0.66	0.63	0.69	0.65	0.63	0.65	0.71	0.9	0.9	0.93	0.79	0.98	0.94	
EBV140_80	0.67	0.58	0.63	0.57	0.71	0.69	0.68	0.7	0.72	0.89	0.94	0.88	0.82	0.96		0.93
EBV140_75	0.64	0.59	0.62	0.58	0.67	0.66	0.64	0.67	0.7	0.92	0.92	0.93	0.79		0.96	0.99
EBV140_70	0.59	0.43	0.46	0.44	0.62	0.47	0.45	0.49	0.92	0.79	0.83	0.78		0.7	0.74	0.66
EBV100_85	0.55	0.43	0.45	0.43	0.55	0.52	0.48	0.51	0.75	0.98	0.93		0.7	0.95	0.9	0.95
EBV100_80	0.53	0.43	0.47	0.43	0.55	0.51	0.48	0.51	0.78	0.95		0.89	0.77	0.89	0.93	0.86
EBV100_75	0.49	0.41	0.43	0.41	0.52	0.48	0.45	0.48	0.76		0.91	0.98	0.68	0.92	0.89	0.89
EBV100_70	0.45	0.31	0.34	0.33	0.47	0.32	0.33	0.36		0.69	0.76	0.71	0.93	0.69	0.71	0.67
EBV60_85	0.71	0.73	0.76	0.72	0.84	0.92	0.94		0.56	0.69	0.72	0.72	0.64	0.78	0.73	0.76
EBV60_80	0.7	0.69	0.73	0.65	0.88	0.99		0.97	0.35	0.61	0.6	0.66	0.5	0.74	0.66	0.74
EBV60_75	0.72	0.69	0.75	0.65	0.9		0.94	0.91	0.52	0.6	0.68	0.63	0.64	0.7	0.7	0.68
EBV60_70	0.77	0.68	0.74	0.66		0.92	0.85	0.88	0.66	0.67	0.77	0.65	0.78	0.7	0.69	0.65
EBV20_85	0.77	0.98	0.93		0.82	0.8	0.74	0.79	0.54	0.59	0.56	0.68	0.53	0.61	0.62	0.64
EBV20_80	0.79	0.96		0.95	0.83	0.8	0.77	0.82	0.67	0.72	0.69	0.79	0.66	0.72	0.68	0.76
EBV20_75	0.79		0.98	0.98	0.77	0.78	0.69	0.77	0.56	0.61	0.59	0.68	0.59	0.62	0.64	0.66
EBV20_70		0.74	0.76	0.72	0.81	0.87	0.82	0.83	0.48	0.6	0.65	0.62	0.64	0.65	0.68	0.63

(c)

	EBV20_70	EBV20_75	EBV20_80	EBV20_85	EBV60_70	EBV60_75	EBV60_80	EBV60_85	EBV100_70	EBV100_75	EBV100_80	EBV100_85	EBV140_70	EBV140_75	EBV140_80	EBV140_85
EBV140_85	0.22	0.18	0.2	0.21	0.47	0.49	0.49	0.48	0.8	0.8	0.79	0.78	0.99	1	1	
EBV140_80	0.2	0.16	0.19	0.19	0.45	0.47	0.47	0.46	0.79	0.79	0.78	0.77	1	1		1
EBV140_75	0.19	0.16	0.18	0.18	0.44	0.46	0.45	0.44	0.78	0.77	0.77	0.76	1		1	0.99
EBV140_70	0.17	0.14	0.16	0.16	0.42	0.45	0.43	0.42	0.76	0.76	0.75	0.74		1	0.99	0.99
EBV100_85	0.43	0.43	0.44	0.45	0.77	0.78	0.78	0.78	0.99	1	1		0.8	0.81	0.82	0.83
EBV100_80	0.45	0.44	0.46	0.47	0.78	0.79	0.79	0.78	1	1		1	0.81	0.82	0.83	0.84
EBV100_75	0.45	0.45	0.46	0.47	0.77	0.78	0.78	0.78	1		1	0.99	0.82	0.83	0.83	0.84
EBV100_70	0.45	0.44	0.46	0.46	0.77	0.78	0.77	0.77		1	1	0.99	0.83	0.84	0.85	0.85
EBV60_85	0.76	0.76	0.76	0.77	0.99	1	1		0.8	0.73	0.74	0.76	0.61	0.62	0.62	0.62
EBV60_80	0.76	0.76	0.77	0.77	1	1		1	0.79	0.73	0.75	0.76	0.61	0.62	0.62	0.62
EBV60_75	0.75	0.76	0.76	0.77	1		1	0.99	0.79	0.72	0.74	0.76	0.61	0.62	0.62	0.62
EBV60_70	0.75	0.75	0.76	0.76		1	1	0.99	0.79	0.73	0.75	0.77	0.61	0.62	0.62	0.62
EBV20_85	0.99	1	1		0.83	0.84	0.84	0.85	0.62	0.52	0.54	0.59	0.52	0.52	0.53	0.53
EBV20_80	1	1		1	0.83	0.84	0.85	0.86	0.63	0.53	0.56	0.61	0.55	0.55	0.56	0.56
EBV20_75	1		1	1	0.83	0.84	0.84	0.85	0.61	0.52	0.55	0.6	0.55	0.54	0.55	0.55
EBV20_70		1	1	0.99	0.83	0.84	0.84	0.85	0.61	0.51	0.54	0.59	0.52	0.52	0.53	0.53

(d)

	EBV20_70	EBV20_75	EBV20_80	EBV20_85	EBV60_70	EBV60_75	EBV60_80	EBV60_85	EBV100_70	EBV100_75	EBV100_80	EBV100_85	EBV140_70	EBV140_75	EBV140_80	EBV140_85
EBV140_85	0.47	0.49	0.49	0.51	0.67	0.67	0.67	0.68	0.91	0.9	0.89	0.89	0.98	0.99	1	
EBV140_80	0.47	0.5	0.5	0.51	0.66	0.66	0.67	0.68	0.9	0.9	0.88	0.87	0.99	1		1
EBV140_75	0.47	0.5	0.5	0.52	0.65	0.65	0.66	0.67	0.88	0.88	0.87	0.86	1		0.99	0.98
EBV140_70	0.45	0.48	0.48	0.49	0.63	0.63	0.63	0.65	0.86	0.86	0.85	0.83		0.99	0.98	0.96
EBV100_85	0.58	0.61	0.62	0.65	0.82	0.83	0.84	0.85	0.99	0.99	1		0.87	0.89	0.92	0.94
EBV100_80	0.58	0.6	0.61	0.64	0.82	0.83	0.84	0.85	0.99	1		1	0.87	0.89	0.92	0.94
EBV100_75	0.57	0.6	0.6	0.63	0.81	0.82	0.83	0.84	1		1	0.99	0.89	0.9	0.93	0.94
EBV100_70	0.57	0.6	0.61	0.64	0.81	0.82	0.83	0.84		1	0.99	0.98	0.9	0.92	0.93	0.95
EBV60_85	0.77	0.8	0.81	0.84	0.98	0.99	1		0.86	0.9	0.9	0.91	0.68	0.72	0.78	0.8
EBV60_80	0.78	0.81	0.82	0.85	0.99	1		1	0.87	0.9	0.9	0.91	0.69	0.73	0.77	0.8
EBV60_75	0.79	0.81	0.83	0.85	1		1	0.99	0.85	0.9	0.89	0.9	0.7	0.74	0.76	0.79
EBV60_70	0.79	0.82	0.84	0.86		1	0.98	0.97	0.84	0.89	0.88	0.89	0.69	0.73	0.75	0.77
EBV20_85	0.98	0.99	1		0.92	0.94	0.91	0.91	0.82	0.9	0.89	0.9	0.75	0.77	0.81	0.84
EBV20_80	0.99	1		1	0.91	0.93	0.9	0.9	0.8	0.89	0.88	0.88	0.73	0.76	0.8	0.83
EBV20_75	1		0.99	0.99	0.92	0.92	0.88	0.88	0.78	0.87	0.87	0.86	0.72	0.75	0.79	0.82
EBV20_70		0.99	0.98	0.98	0.89	0.89	0.84	0.84	0.74	0.85	0.84	0.84	0.7	0.72	0.76	0.8

Figure 6 – Spearman's rank correlation (ρ) for the best 5% sires (above diagonal) and 5% females (below diagonal) for estimated breeding value (EBV) for Total Oocytes (a), Viable Oocytes (b), Cleaved Embryos (c), Viable Embryos (d) by four ages at ovum pick-up (AOPU) 20, 60, 100 and 140 months in four different temperature-humidity index (THI) 70, 75, 80, and 85.

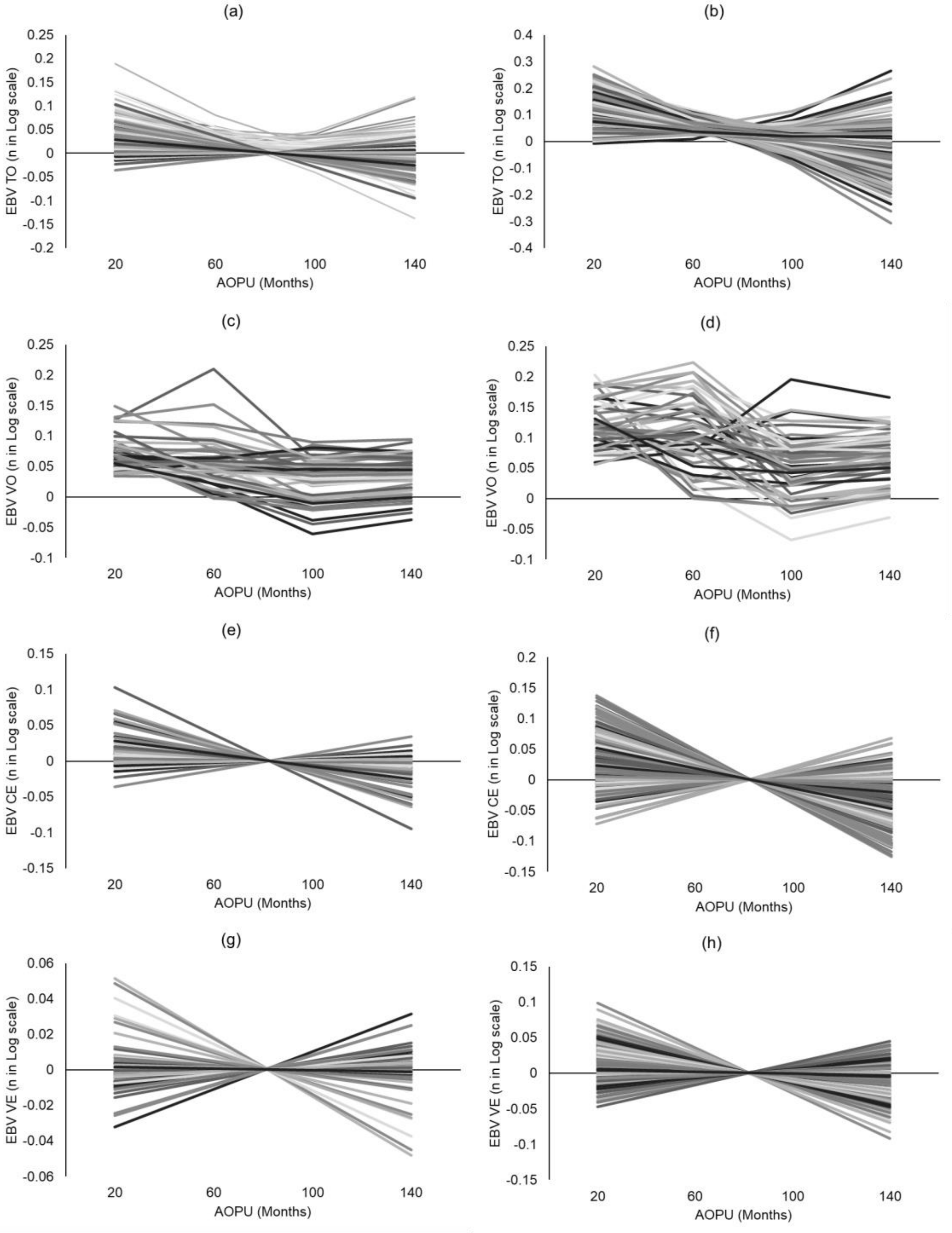


Figure 7 – Estimated breeding values (EBV) of the 5% best sires (a, c, e, and g) and 5% best females (b, d, f, and h) (selected by EBV in 20 months at THI equal 80) for Total Oocytes (a, and b), Viable Oocytes (c, and d), Cleaved Embryos (e, and f), Viable Embryos (g, and h) in four different age at ovum pick-up (AOPU: 20, 60, 100, and 140 months).

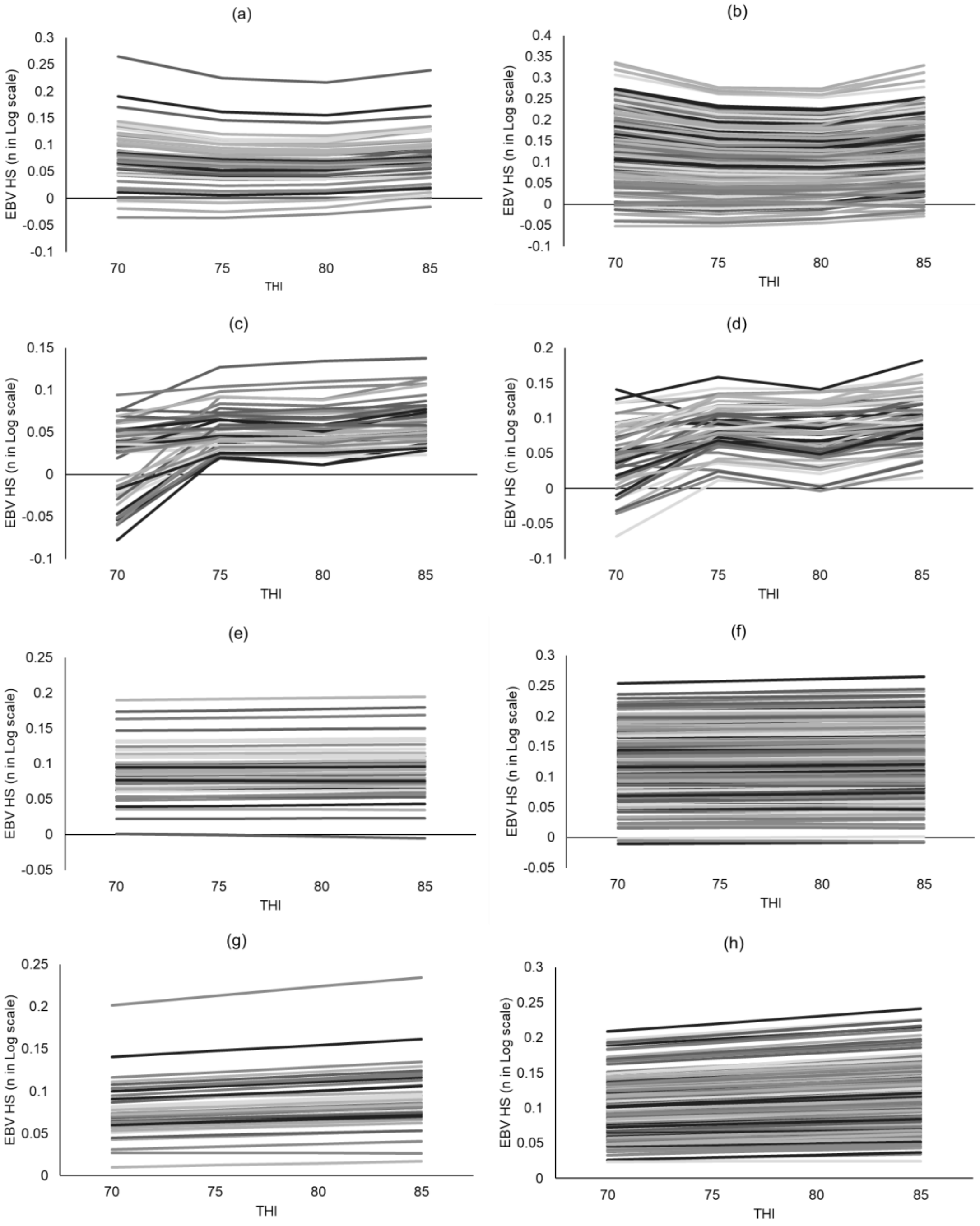


Figure 8 – Estimated breeding values (EBV) of the 5% best sires (a, c, e, and g) and 5% best females (b, d, f, and h) (selected by EBV in 20 months at THI equal 80) for Total Oocytes (a, and b), Viable Oocytes (c, and d), Cleaved Embryos (e, and f), Via Viable Embryos (g, and h) in EBV for heat stress (HS) in four different temperature-humidity index (THI: 70, 75, 80, and 85).

CAPÍTULO IV

Comparing Bayesian models for genetic evaluation of oocytes and embryo counts in Gir dairy cattle[5]

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Comparing Bayesian models for genetic evaluation of oocytes and embryo counts in Dairy Gir cattle

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ABSTRACT

Count traits are usually explored in livestock breeding programs and it usually does not fit into Normal distribution, requiring alternatives to adjust the phenotype to estimate accurate genetic parameters and breeding values. Alternatively, distribution such as Poisson can be used to evaluate count traits. This study aimed to compare and discuss the genetic evaluation for oocyte and embryo counts considering Gaussian (untransformed variable – LIN, transformed by logarithm – LOG, transformed by Anscombe – ANS) and Poisson (POI) distributions. The data comprised 11343 total oocytes, viable oocytes, cleaved embryos, viable embryos records of ovum pick-up from 1740 dairy Gir heifers and cows. The genetic parameters and breeding values were estimated by the *MCMCglmm* package of the R software. The posterior means of heritability varied from 0.40 (LIN) to 0.49 (POI) for TO; 0.39 (LIN) to 0.49 (POI) for VO; 0.30 (LOG) to 0.41 (POI) for CE; 0.19 (LIN) to 0.32 (POI) for VE, respectively. The posterior means of repeatability varied from 0.56 (LIN) to 0.65 (POI) for TO; 0.53 (LOG) to 0.63 (POI) for VO; 0.44 (LOG) to 0.60 (POI) for CE; 0.36 (LOG) to 0.56 (POI) for VE, respectively. Deviance information criterion and mean squared residuals indicated that POI model should be used for genetic evaluation of embryo and oocytes count traits. The Spearman rank correlation between estimated breeding value (EBV) for embryo and oocytes count traits computed by POI, LOG, and ANS models was high (ranging from 0.77 to 0.99), indicating little reordering among the best animals. The POI model is the most adequate for genetic evaluation, resulting in more reliable EBV for Dairy Gir cattle of oocyte and embryo counts traits.

Keywords: dairy cattle, breeding, *Bos Indicus*, *in vitro* fertilization.

1. Introduction

Artificial reproductive technologies might contribute to increasing the selection intensity, reducing the number of selected animals and generation intervals (Panetto et al., 2010). Moreover, it is well known in the literature that it is possible to obtain genetic progress of traits related to reproductive technologies through selection. For instance, the estimated heritability and repeatability of number of viable embryos ranged from low (0.03 and 0.13) to moderate (0.21 and 0.34), respectively (Asada and Terawaki, 2002; Jaton et al., 2016a; König et al., 2007; Merton et al., 2009; Tonhati et al., 1998). Therefore, genetic selection will help the identification and selection of cows most likely to respond favorably to reproductive procedures such as superovulation and in vitro fertilization.

Counting data, such as those collected in artificial reproduction techniques, usually do not present a normal distribution and some authors described that using square root and logarithm adjustment function will result in more accurate predictions (Jaton et al., 2016a; Parker Gaddis et al., 2017). While other author evaluated alternatives distributions such as Poisson to estimate accurate EBV for count data (Merton et al., 2009). For instance, the Poisson distribution was used to estimate genetic parameters of tick counting (Ayres et al., 2013), black spots on wool (Naya et al., 2008) and number of piglets born and weaned (Ventura et al., 2015) as well as for artificial reproduction traits in Guzerá cows (Perez et al., 2017b). It is important to highlight that higher heritability and repeatability values were reported by Poisson distribution when compared to untransformed data or logarithmic transformation. However, low reordering of the top candidates were observed across linear and Poisson distribution (Perez et al., 2017b). It is important to note that, models that consider logarithmic and Anscombe transformation, and Poisson distribution have not yet been evaluated for artificial reproduction traits in dairy Gir cattle.

The objective of this study was to compare and discuss the genetic parameters and estimated breeding values for oocyte and embryo counts considering Gaussian (untransformed variable – LIN, transformed by logarithm – LOG, transformed by Anscombe – ANS) and Poisson (POI) distributions and determine the best approach for genetic evaluation of oocyte and embryo counts in Brazilian Dairy Gir Cattle.

2. Material and methods

The data used were from three herds of Dairy Gir cattle, belonging to the same company, in the state of Minas Gerais – Brazil (19º55' S – 43º57' W). The available information was on traits of total oocytes (TO), viable oocytes (VO), cleaved embryos (CE), and viable embryos (VE), for this study.

The concatenation of herd, year, and ovum pick-up season defines the contemporary groups for all traits. Seasons were classified into wet (October until March) or dry (April until September). Contemporary groups that contained fewer than five observations were eliminated. For CE and VE traits, service sires used only once have been eliminated.

Following these criteria, 11,343 (TO, VO) and 11,305 (CE, VE) records of ovum pick-up from 1,740 and 1,734 Dairy Gir heifers and cows from three farms, collected from 2008 to 2017, were analyzed. Semen from 176 different sires (Gir or Holstein) was used in *in vitro* fertilization procedures. We used the same database for all models evaluated. The pedigree file included 5,908 animals; the summary of phenotypic data is available in Table 1.

The data editing to compute descriptive statistics and the test of significance of fixed effects that were included in the mixed model for genetic analysis were performed in R software (R Core Team, 2017).

The first model (LIN) assumed Gaussian distribution with untransformed data. The second model (LOG) assumed Gaussian distribution with common logarithm transformed data: $y_i = \log(y + 1.001)$ where y_i is the log transformed value of oocyte and embryo count and y e the original value of oocyte and embryo count. The third model (ANS) assumed Gaussian distribution with Anscombe transformed data: $y_i = 2 * \sqrt{\left(y + \frac{3}{8}\right)}$ where y_i is the Anscombe transformed value of oocyte and embryo count and y e the original value of oocyte and embryo count. The fourth model assumed Poisson distribution (POI).

Genetic parameters and breeding values were estimated using the following univariate Bayesian model:

$$y_{ijk} = hys_i + iop_j + nop_j + aop_j + u_j + pe_j + sa_k + e_{ijk},$$

in which y_{ijk} is the vector of trait value (TO, VO, CE, and VE); hys_i is the fixed effect of herd-year-season of ovum pick-up; iop_j is the covariable effect of the interval between ovum pick-up in the same animal; nop_j is the covariable effect of animal's ovum pick-up number; aop_j is the covariable effect of animal's age at ovum pick-up; sa_l fixed effect referring to sire used in artificial fertilization (only used for CE and VE); u_j and pe_j are the additive genetic and permanent environment effects on the performance of animal j; and e_{ijk} is the random error.

For which we assume:$Var\begin{bmatrix} \boldsymbol{u} \\ \boldsymbol{pe} \\ \boldsymbol{e} \end{bmatrix} = \begin{bmatrix} \mathbf{A}\sigma_a^2 & 0 & 0 \\ 0 & \boldsymbol{I}\sigma_{pe}^2 & 0 \\ 0 & 0 & \boldsymbol{I}\sigma_e^2 \end{bmatrix}$, in which where σ_a^2 is the additive genetic variance, ***A*** the numerator relationship matrix, σ_{pe}^2 the variance of permanent effects of the donor, σ_e^2 the residual variance and ***I*** represents an identity matrix.

Analyzes were performed in the *MCMCglmm* package (Hadfield and Nakagawa, 2010) from R software (R Core Team, 2017), 1,100,000 samples were generated, assuming a burn-in period of 100,000 and a thinning interval of 50. Except for TO in models LIN, ANS and POI and for VO in model POI. In these cases, the number of samples, burn-in and thinning interval were 1,300,000; 300,000 and 50, respectively. Thus, in all cases, the inference was realized over 20,000 samples from the posterior distribution. Convergence of the Markov Chain Monte Carlo chains was verified by tests of Geweke (1991), Raftery and Lewis (1991), and Heidelberger and Welch (1983) using the *coda* package (Plummer et al., 2006) from R software (R Core Team, 2017).

The deviance information criteria (DIC) and mean squared error (MSE) were used to identify the model of best fit to embryo and oocyte count traits. The DIC was used to compare LIN and POI models only, because the LOG and ANS models considered a response variable on a logarithmic and normalized scales, respectively. It was estimated as $DIC = \overline{D}(\theta) + P_D = 2\overline{D}(\theta) + D(\bar{\theta})$ where $\overline{D}(\theta) = E_{0|y}[\overline{D}(\theta)]0$ is the posterior expectation of Bayesian deviance and $D(\theta) = -2\log p(y|0)$ corresponds to the goodness of fit of the model (Spiegelhalter et al., 2002). Lower values of DIC indicate better goodness of fit.

The MSE was used in all models. MSE = $\frac{\sum(y_j - \hat{y}_j)^2}{n}$, where $\hat{y}_j$ is the value estimates of trait by models; y_j the value of the corresponding observation, n the number of observations.

Because some of the predicted values were on different scales for the different models, they were back transformed to the original scale (probability) using the inverse link function. For LOG, $\hat{y}_j$: was back transformed to the original scale (embryo/oocyte count) by $10^{\hat{y}_j + 1.001}$ and the other calculations were performed thereafter. In ANS model: $\hat{y}_j = \left(\frac{y}{2}\right)^2 - \frac{3}{8}$, in POI model: $\hat{y}_j = \exp(\hat{\eta} + (1/2)\sigma_e^2)$. Except for the linear models because: $\hat{y}_j = \hat{\eta}$.

The Spearman’s ranking correlation between the EBV for embryo and oocytes count traits of the top 5% sires and females were calculated to verify the occurrence of changes in the ranking of the best animals at different models.

Approximate EBV reliabilities were calculated for traits in a Bayesian approach as $Rel \ = 1 - \sigma(u_i)^2/((1 + f) * (\bar{x}\ (\sigma_u^2))$, where σ is standard deviation, x is mean, f is inbreeding coefficient (Aguilar et al., 2020), u_i is the posterior distribution for the *ith* animal solution and σ_u^2 is the posterior distribution for the additive genetic variance. Here we considered $\sigma(u_i)^2$ as equivalent to the prediction error variance (PEV) commonly used in the frequentist, approach, similarly to Vizoná et al. (2020).

3. Results

The posterior means and quantiles (0.025 and 0.975, in parentheses) of heritability ranged from 0.40 (0.31 and 0.48, LIN) to 0.49 (0.33 and 0.50, POI) for TO; 0.39 (0.30 and 0.47, LIN) to 0.49 (0.39 and 0.58, POI) for VO; 0.30 (0.22 and 0.39, LOG) to 0.41 (0.30 and 0.51, POI) for CE; 0.19 (0.12 and 0.27, LIN) to 0.32 (0.21 and 0.44, POI) for VE, respectively (Table 2).

The posterior means and quantiles of repeatability showed wide variation across models, especially for CE and VE (Table 2). For instance, the posterior means of repeatability ranged from 0.56 (0.53 and 0.58, LIN) to 0.65 (0.62 and 0.68, POI) for TO; 0.53 (0.50 and 0.56, LOG) to 0.63 (0.61 and 0.66, POI) for VO; 0.44 (0.41 and 0.47, LOG) to 0.60 (0.56 and 0.63, POI) for CE; 0.36 (0.33 and 0.39, LOG) to 0.56 (0.52 and 0.59, POI) for VE, respectively.

The posterior distribution of both heritability and repeatability of all evaluated traits overlapped across models, showing that no significant differences were identified.

The POI models presented the smallest DIC values showing that the POI models presented the best goodness of fit for all evaluated traits (Figure 1) with values of -15.6% for TO, -17.3% for VO, -14.2% for CE, and -18.7% for VE.

POI and LIN models showed lower MSE values for all traits when compared to ANS and LOG models. The high MSE estimates related to ANS and LOG models might be partly attributed to some bias caused by the data transformation (Figure 2). The LIN model presented the lowest MSE values for TO and VO, while for CE the MSE values related to LIN and POI models were similar and for VE the POI model showed lowest MSE value.

The Spearman rank correlation among EBV of the top 5% sires and females for embryo and oocytes count traits computed by POI, LOG and ANS models were high, ranging from 0.77 to 0.99, indicating small reordering among the top 5% sires and females (Figure 3). On the other hand, small Spearman rank correlation ranging from 0.39 to 0.85 for top 5% males and from 0.75 to 0.94 for top 5% females were observed between LIN and other (ANS, LOG and POI) models. Thus, the linear model should not be used to genetic evaluation of oocyte and embryo counts trait as the selection of candidates can be negatively impacted when the data transformation or alternative distribution, such as POI model is not used.

The posterior means of reliability of EBV of donors in all traits was slightly higher when using the POI model, indicating that this model results in more accurate EBV than the other models (Table 3). In the case of the EBV of sires with at least five daughters with ovum pick-up, the reliability was very similar between the ANS, LOG and POI models, however, the reliability estimated by LIN model was slightly lower than all other evaluated models.

Estimated breeding values ranged between -1.16 and 1.27 for TO, between -1.07 and 1.27 for VO, between -0.95 and 0.85 for CE, and between -1.24 and 0.77 for VE, indicating that there is enough genetic variability for genetic selection of sires for all traits. A total of 601 (56.48%), 571 (53.66%), 548 (51.60%), and 513 (48.30%) sires and 2,538 (52.34%), 2,431 (50.18%), 2,368 (49.03%), and 2,198 (45.50%) females had positive EBV, of TO, VO, CE, and VE, respectively. As well as 19 (1.78%), 18 (1.69%), 12 (1.13%), and 12 (1.13%) sires and 971 (20.04%), 916 (18.91%), 551 (11.41%), and 134 (2.77%) females had positive EBV and reliability above 0.60, indicating the existence of feasibility for the implementation of selection procedures in the population in order to improve reproductive efficiency for these traits in future generations, meeting the demand of breeders who sell embryos and the artificial reproduction industry.

4. Discussion

In general, the posterior means of heritability and repeatability of embryo and oocytes traits presented here were higher than those reported in the literature. The fact that the farms present similar conditions, located in the same region, and have the same technical support and animal husbandry techniques, which standardizes management and highlights genetic differences between animals might partially explain those differences. The occurrence of pre-selection of females that will undergo both *in vitro* and *in vivo* production of embryos is a factor that can result in lower heritability (Vizoná et al., 2020), as in the case of studies using MOET (multiple ovulation and embryo transfer).

The heritability estimates for TO range from 0.13 to 0.31 in Holstein cows raised in Netherlands and the United States, respectively (Cornelissen et al., 2017; Parker Gaddis et al., 2017). These values are smaller than the ones found in our study, and both used LOG and ANS transformed data. The heritability estimates for VO ranges from 0.16 (untransformed date) to 0.25 (Poisson model) in Brazilian Guzerá Breed (Perez et al., 2017). While in Brazilian dairy Gir the heritability estimate for VO was 0.32 when using Poisson distribution (Vizoná et al., 2020). The heritability estimates for CE ranged from 0.13 to 0.19 using linear, logarithm transformed and Poisson models in Brazilian Guzerá Breed (Perez et al., 2017b). Similarly the heritability estimates for VE varied from 0.11 to 0.20 when using linear (untransformed) and Poisson models (Perez et al., 2017b), and 0.27 when using Poisson model (Vizoná et al., 2020).

The LIN model resulted in the lowest posterior means of heritability for VO, and VE and, similar results were reported by Perez et al. (2017). Moreover, these authors reported highest genetic effects for number of viable oocytes, number of grade I oocytes, number of degenerated oocytes, number of cleaved embryos and number of viable produced embryos were estimated by POI model when compared to LIN, and LOG. However, we observed overlapped distribution of heritability estimates for TO, VO, CE, and VE when using ANS, LIN, LOG, and POI models, in our study.

The moderate to high posterior means of heritability for oocyte and embryo count traits reported in this study indicated that selection can result in rapid genetic progress, especially when using Poisson distributions.

The repeatability estimates ranging from 0.40 to 0.57 for TO (Parker Gaddis et al., 2017), 0.32 to 0.63 for VO (Perez et al., 2017b, 2016), 0.27 to 0.55 for CE (Parker Gaddis et al., 2017; Perez et al., 2017, 2016), and 0.21 to 0.51 for VE (Bényei et al., 2004; Perez et al., 2017b) is available on the literature. In general, higher repeatability values are reported for oocytes when compared to embryos, it might be partially explained by the effects of service sire and laboratory techniques. Moreover, repeatability for total number of embryos and number of viable embryos traits estimated by logarithm transformed model were smaller than the values obtain by Anscombe transformation (Jaton et al., 2016a). While the repeatability estimated for VO by logarithmic transformation model was higher than the values obtained by untransformed variable model (Perez et al., 2017). The posterior means of heritability and repeatability for the number of embryos estimated by untransformed data model resulted in lower and higher values, respectively, than the values estimated by logarithm transformed model (Bényei et al., 2004). Therefore, the results obtained here and those published in the literature suggested that logarithmic transformation is less efficient in capturing the permanent environment variance than the untransformed data, Anscombe transformation and Poisson model.

The high posterior means of repeatability estimated here indicated that the donors should maintain the oocytes and embryo count constant over time, in the different ovum pick-up events. Therefore, young females with high oocyte and embryo count at their first ovum pick up event should be selected as donors for artificial reproductive techniques (Vizoná et al., 2020).

The small Spearman rank correlations between the EBV of the top 5% sires and females estimated by LIN and all other models (ANS, LOG, and POI) indicated considerable rearrangement of top candidates can occur when using the LIN model. The Spearman rank correlation between EBV for the number of piglets born and weaned estimated by Poisson and Gaussian distribution presented high values, above 0.8 (Ventura et al., 2015). Similarly, the Spearman correlation above 0.86 and 0.92 were estimated for tick count (Ayres et al., 2013) and for *in vitro* embryo production traits (Perez et al., 2017a), respectively. Thus, our results demonstrate the importance of data transformation, and/or the use of a model that considers the Poisson distribution in the genetic evaluation of oocyte and embryo counts.

High Spearman rank correlation among the EBV of the top 5% sires and females were estimated across LOG, ANS, and POI models, demonstrating small reranking of the top candidates when using Logarithmic and Anscombe demonstrate that there are little reranking of the best animals when using Logarithmic transformation, Anscombe or Poisson distribution models. It is an important result as the majority of genetic parameters for embryo count published in the last 10 years were estimated by logarithmic or Anscombe transformation (Cornelissen et al., 2017; Jaton et al., 2016a, 2016c; Parker Gaddis et al., 2017; Perez et al., 2016) and only a few studies used Poisson distribution models (Perez et al., 2017b; Vizoná et al., 2020).

Reliability is an important parameter in breeding programs as it also measures the potential for response to selection (Gorjanc et al., 2015). Our results showed small changes in the EBV reliability for sires with at least five daughters with ovum pick-up in TO, VO, and CE when Gaussian or Poisson distributions were used. On the other hand, large changes in the EBV reliability for females with ovum pick-up was observed whit Poisson distribution. It might be partially explained by the strong relationship between the additive genetic and phenotypic variances as the precision of EBV is measured by relating the PEV of EBV to the additive genetic variance of population. Thus, highlighting the advantages of using a model that considers the Poisson distribution for total oocytes, viable oocytes, cleaved embryos, and viable embryos.

The EBV estimates reported here demonstrated the existence of considerable genetic variability for oocytes and embryo counts traits, confirming that selection of donors that best responded to the technique should increase the genetic gain of oocytes and embryo counts through selection across generations. The selection for the production of oocytes and embryos might not be a general selection objective for commercial herds (Jaton et al., 2016a), however it can be useful for elite and multipliers herds. For instance, since 2019 1,419,336 transferable embryos were produced worldwide (IETS, 2020), which reinforces the importance of these traits for livestock industry.

5. Conclusions

A better performance of oocytes and embryos production in the dairy Gir cattle can be achieved from the genetic selection due to the genetic variation detected, especially if the genetic evaluation model considers the Poisson distribution in the traits to allow the prediction of breeding values more accurate.

The selection of donors after the first ovum pick-up for oocytes and embryo count traits in Dairy Gir cattle, can be implemented due to the amount of repeatability reported. In this way, it is possible to reduce the number of ovum pick-up in donors that have low production in the first ovum pick-up, reducing costs and time, and obtaining greater number of oocytes and embryos per ovum pick-up, increasing the profitability of the technique.

The selection of donors after the first ovum pick-up for oocyte and embryo count traits in Dairy Gir cattle can be implemented due to the magnitude of genetic variability, repeatability and reliability in predicting the breeding values of females. In a practical way, these facts indicate that it is possible to reduce the number of ovum pick-up in donors who have low production in the first ovum pick-up (reducing costs and time), as well as increase the number of oocytes and embryos per ovum pick-up in more efficient females, additionally contributing to improvement in the profitability of the use of the technique in the population.

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Table 1 – Number of observations (N), medians, means, standard deviations (SD), Minimum and maximum (untransformed variables).

Trait	Mean	Median	SD	Max	Min
TO	21.71	18	15.27	157	1
VO	16.25	13	12.91	142	1
CE	12.01	10	8.85	98	0
VE	4.61	3	4.58	43	0

Total Oocytes (TO), Viable Oocytes (VO), Cleaved Embryos (CE), and Viable Embryos (VE).

Table 2 – Estimates of mean and quantiles (0.025, 0.975) of the posterior distribution of the heritability (h2) and repeatability (R) for the traits and models studied.

Trait	Model	Heritability (h^2)			Repeatability (R)		
		Mean	0.025	0.975	Mean	0.025	0.0975
TO	ANS	0.42	0.33	0.51	0.58	0.55	0.61
TO	LIN	0.40	0.31	0.48	0.58	0.55	0.60
TO	LOG	0.42	0.33	0.50	0.56	0.53	0.58
TO	POI	0.49	0.39	0.59	0.65	0.62	0.68
VO	ANS	0.41	0.33	0.50	0.56	0.53	0.59
VO	LIN	0.39	0.30	0.47	0.56	0.53	0.58
VO	LOG	0.41	0.32	0.49	0.53	0.50	0.56
VO	POI	0.49	0.39	0.58	0.63	0.61	0.66
CE	ANS	0.32	0.24	0.41	0.48	0.45	0.51
CE	LIN	0.31	0.23	0.40	0.48	0.45	0.51
CE	LOG	0.30	0.22	0.39	0.44	0.41	0.47
CE	POI	0.41	0.30	0.51	0.60	0.56	0.63
VE	ANS	0.21	0.13	0.29	0.38	0.35	0.41
VE	LIN	0.19	0.12	0.27	0.37	0.34	0.40
VE	LOG	0.20	0.12	0.28	0.36	0.33	0.39
VE	POI	0.32	0.21	0.44	0.56	0.52	0.59

Total Oocytes (TO), Viable Oocytes (VO), Cleaved Embryos (CE), and Viable Embryos (VE). Anscombe transformation model (ANS), Linear model (LIN), Logarithmic transformation model (LOG), and Poisson model (POI).

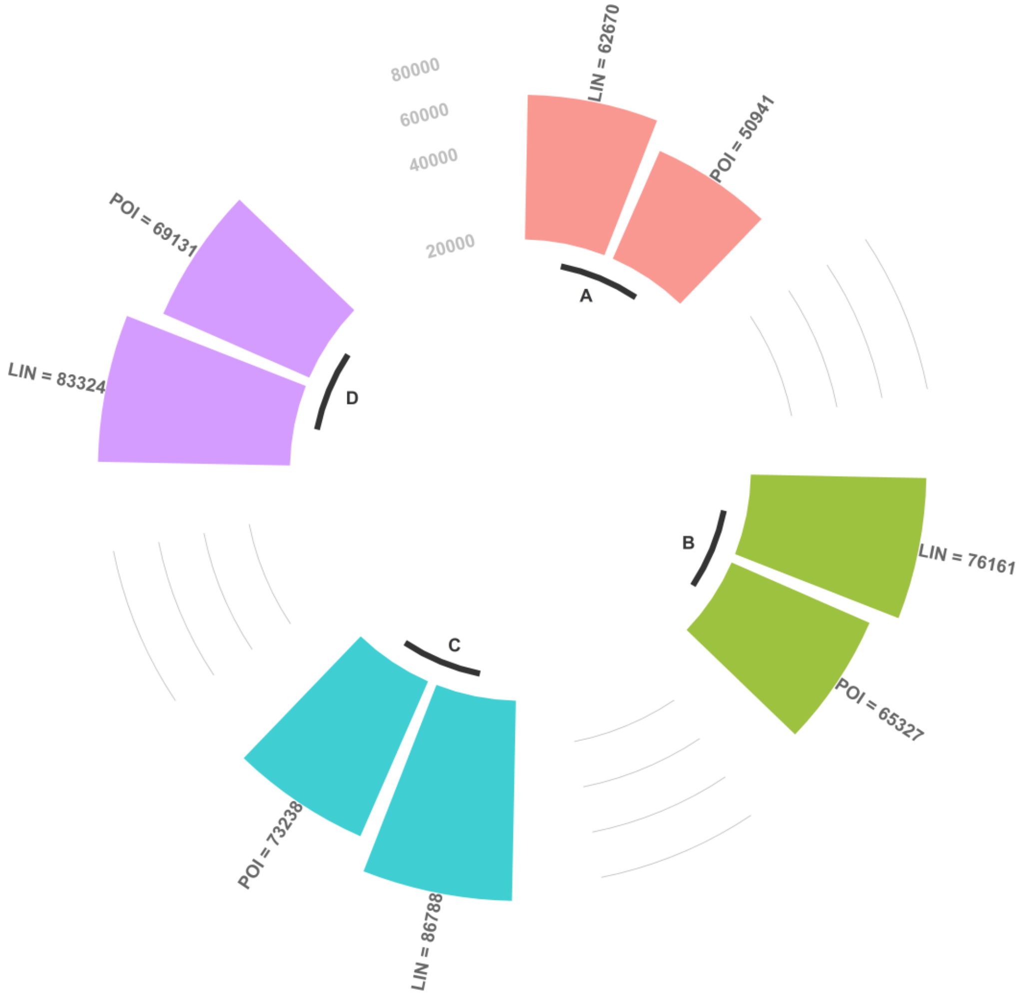

Figure 1 – Estimated deviance information criterion (DIC) for total oocytes (A), viable oocytes (B), cleaved embryos (C), and viable embryos (D) in linear model (LIN) and Poisson model (POI).

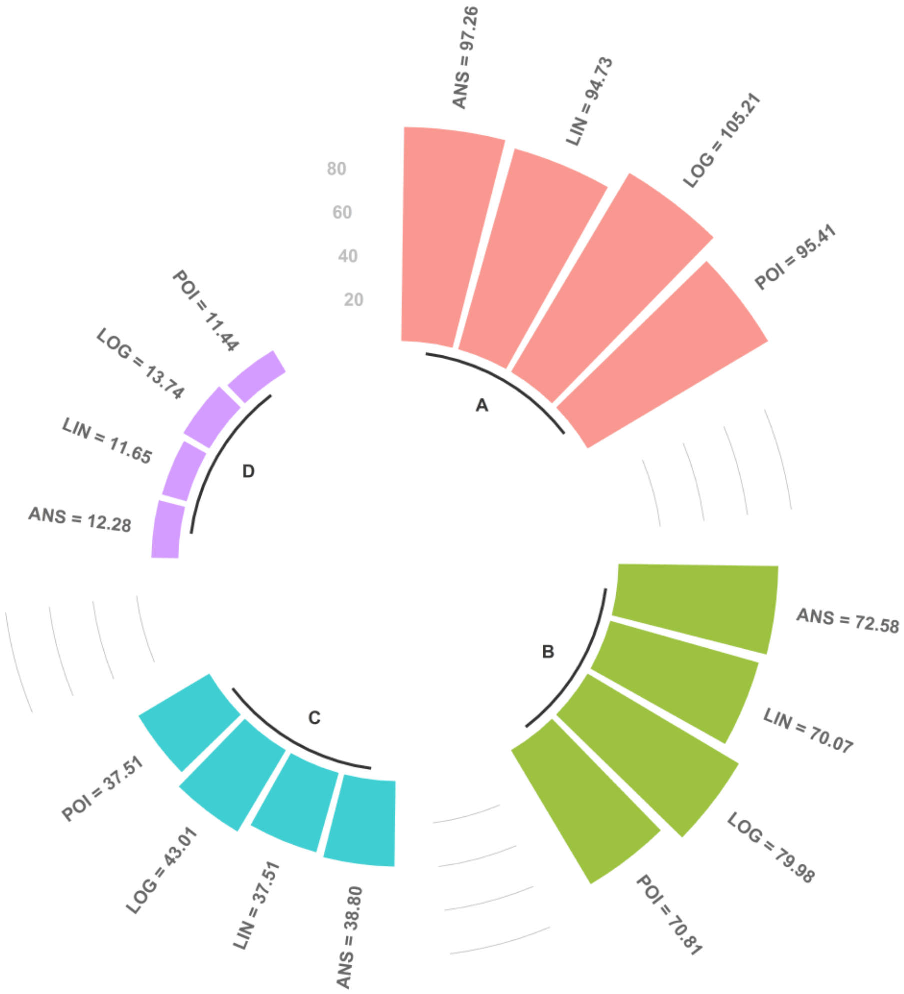

Figure 2 – Mean squared error (MSE) between observed and fitted values for total oocytes (A), viable oocytes (B), cleaved embryos (C) and viable embryos (D) in Anscombe transformation (ANS), linear model (LIN), logarithmic transformation (LOG) and Poisson model (POI).

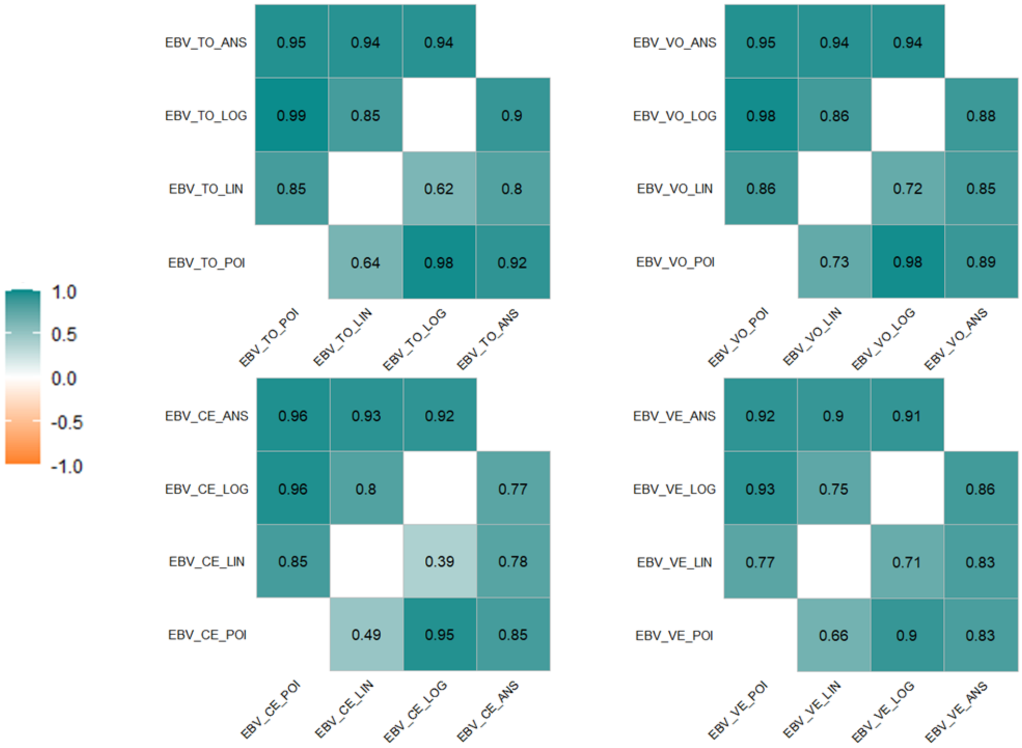

Figure 3 – Spearman's rank correlation for the best 5% sires (below diagonal) and 5% females (above diagonal) for estimated breeding value (EBV) for total oocytes (TO) viable oocytes (VO) cleaved embryos (CE) and viable embryos (VE) in Anscombe transformation (ANS), Linear model (LIN), Logarithmic transformation (LOG) and Poisson model (POI).

Table 3 – Reliability of estimated breeding values (EBV) for total oocytes (TO) viable oocytes (VO) cleaved embryos (CE) and viable embryos (VE) in Anscombe transformation (ANS), Linear model (LIN), Logarithmic transformation (LOG) and Poisson model (POI).

Trait	Model	Donors*	Sire's father of donors**
TO	ANS	0.66 (0.43 – 0.87)***	0.75 (0.49 – 0.95)
TO	LIN	0.64 (0.41 – 0.86)	0.74 (0.47 – 0.95)
TO	LOG	0.67 (0.43 – 0.88)	0.75 (0.48 – 0.95)
TO	POI	0.68 (0.45 – 0.88)	0.75 (0.48 – 0.96)
VO	ANS	0.67 (0.42 – 0.87)	0.75 (0.49 – 0.95)
VO	LIN	0.64 (0.40 – 0.86)	0.74 (0.47 – 0.95)
VO	LOG	0.67 (0.42 – 0.88)	0.75 (0.49 – 0.95)
VO	POI	0.68 (0.44 – 0.88)	0.75 (0.49 – 0.95)
CE	ANS	0.60 (0.32 – 0.85)	0.72 (0.43 – 0.94)
CE	LIN	0.59 (0.21 – 0.84)	0.71 (0.42 – 0.94)
CE	LOG	0.60 (0.32 – 0.85)	0.72 (0.42 – 0.94)
CE	POI	0.61 (0.30 – 0.85)	0.72 (0.49 – 0.95)
VE	ANS	0.50 (0.23 – 0.78)	0.67 (0.34 – 0.93)
VE	LIN	0.48 (0.18 – 0.77)	0.65 (0.32 – 0.93)
VE	LOG	0.50 (0.23 – 0.78)	0.67 (0.34 – 0.93)
VE	POI	0.52 (0.19 – 0.19)	0.68 (0.35 – 0.93)

* Females with ovum pick-up.
** Sires with at least five daughters with ovum pick-up.
*** Minimum and maximum

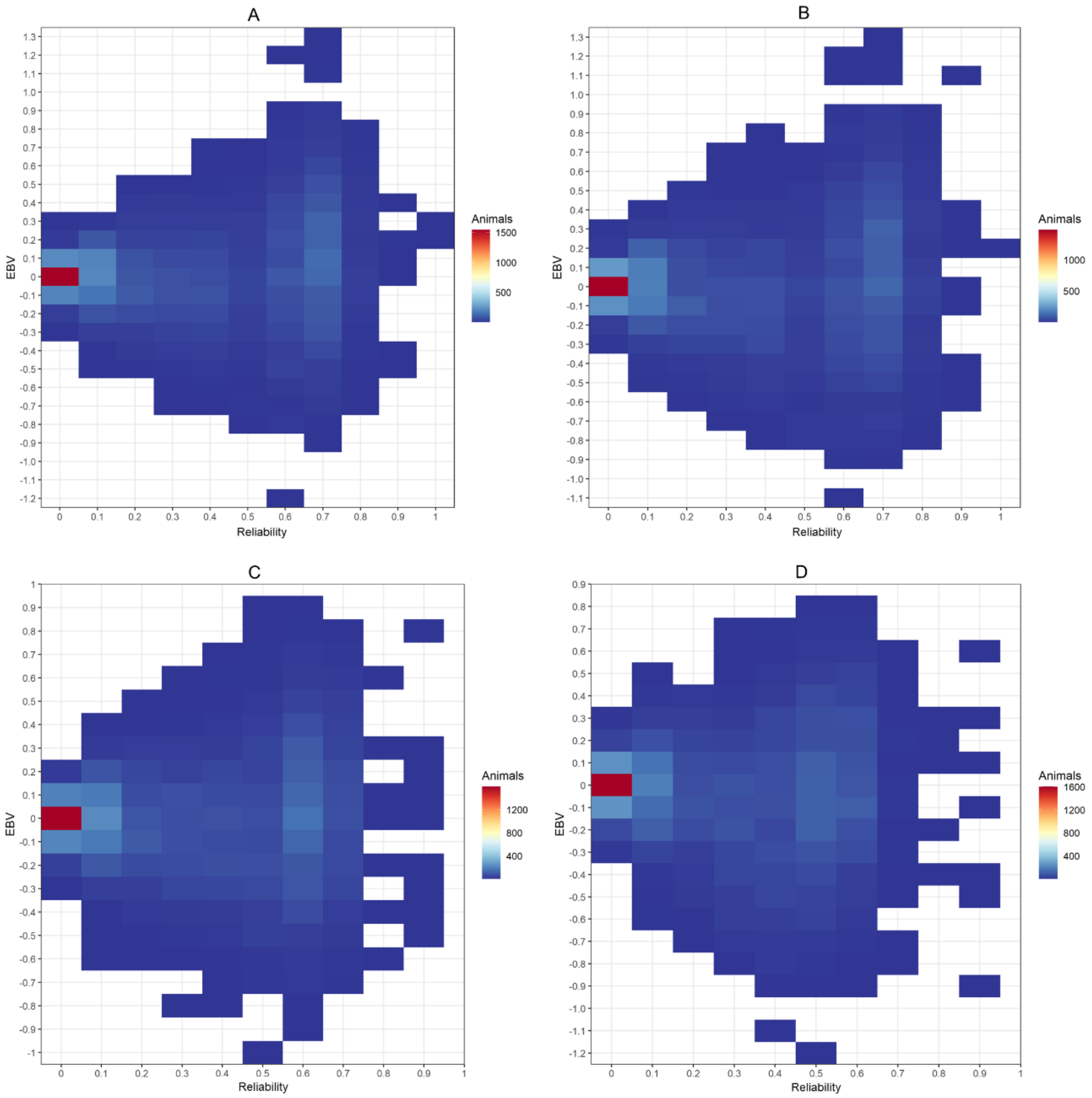

Figure 4 – The joint distribution of reliabilities and estimated breeding values (EBV) for all animals for traits of total oocytes (a), viable oocytes (b), cleaved embryos (c), viable embryos (d) using Poisson model for genetic evaluation in dairy Gir cattle.

5. Considerações finais

As características de reprodução artificial têm grande potencial para seleção genética, o que pode resultar em um rápido progresso genético e, como consequência, aumentar a taxa reprodutiva de fêmeas com maior interesse comercial, como aquelas que têm alto valor genético para características economicamente importantes aos diferentes sistemas de produção e a uma raça com tamanha expressividade no país.

A utilização do modelo de regressão aleatória em substituição ao modelo de repetibilidade na avaliação genética de oócitos totais, oócitos viáveis, embriões clivados e embriões viáveis aumenta o ganho genético nessas características na raça Gir Leiteiro, e o mesmo pode ser esperado em outras raças.

As características de reprodução artificial dessa população apresentam altas estimativas de repetibilidade, indicando que contagens de oócitos e embriões semelhantes devem se repetir em diferentes momentos na vida do animal e que a seleção de animais pode ser realizada de forma acurada após ter o resultado da primeira coleta de óocitos.

A confiabilidade do valor genético obtida pelo modelo de regressão aleatória é maior nos períodos em que ocorre o maior número de coleta. Portanto, aumentar o número de coletas em fêmeas jovens aumenta a confiabilidade do valor genético predito em idade precoce.

O índice de temperatura e umidade deve ser considerado na estimativa de parâmetros genéticos e valores genéticos previstos para produção de oócitos totais e viáveis e produção de embriões clivados e viáveis em vacas Gir Leiteiro, devido ao fato de afetar produção de oócitos e embriões e a predição dos valores genéticos dessas características.

Estimativas de herdabilidade de magnitude moderada e alta para a produção de oócitos e embriões foram observadas em diferentes idades a coleta de oócitos e índice de temperatura e umidade, indicando que a seleção genética pode resultar em rápido progresso genético. Entretanto, a classificação dos melhores animais alterou entre as diferentes idades a coleta e índice de temperatura e umidade.

A seleção de animais tolerantes ao estresse térmico pode levar a um aumento na taxa reprodutiva artificial das fêmeas com alto mérito genético para distintas características de interesse econômico, mesmo em condições de índice de temperatura e umidade elevado.

A avaliação genética considerando diferentes modelos lineares generalizados sob enfoque bayesiano para características de contagem de oócitos e embriões apresentaram médias posteriores de herdabilidade e repetibilidade de magnitude moderadas a altas na população avaliada de bovinos da raça Gir Leiteiro. O modelo de Poisson apresentou maiores médias posteriores de herdabilidade e repetibilidade e melhor ajuste entre todos os modelos avaliados.

A correlação de rank de Spearman entre os valores genéticos de machos e fêmeas preditos para as características de contagem de oócitos e embriões foi alta entre os modelos que consideraram distribuição de Poisson e que foram transformados por logaritmo e por Anscombe. Os valores genéticos preditos pelo modelo Poisson apresentam maior confiabilidade dos valores genéticos do que os demais. Assim, o modelo que considera a distribuição de Poisson dos registros reprodutivos deve ser utilizado em futuras avaliações genéticas para características de contagem de oócitos e embriões em bovinos Gir Leiteiro.

A disponibilização dos valores genéticos, na forma de PTA (Habilidade Prevista de Transmissão) ou DEP (Diferença Esperada na Progênie), para a produção de oócitos e embriões, pela avaliação genética, é uma medida aconselhável uma vez que essas características têm considerável importância para os rebanhos de elite (núcleo).

Futuras pesquisas que considerem a utilização do modelo de regressão aleatória considerando a distribuição de Poisson dos registros são encorajadas, bem como a utilização de informações genômicas nesses modelos, visando aumentar a confiabilidade dos valores genéticos preditos.

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Vita

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