Bull effects on *in vitro* embryo production quality traits are repeatable and heritable

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Abstract
In this study, *in vitro* production of embryos (IVP) was performed using oocytes aspirated from slaughterhouse ovaries and fertilized with ejaculates from 123 bulls aged between 10- and 92 months at semen collection. The restricted maximum likelihood approach was used to estimate genetic parameters for embryo production quality traits including blastocyst rate, hatch rate and an index trait combining Blastocyst rate, Kinetic Score, and Morphology score (BLxMxK). A total of 259 records were available for analysis. The results indicate that bulls at a younger age had better performances in all the studied IVP traits compared to bulls at later ages. The results also show low to high heritability and moderate to high repeatability for the IVP traits, suggesting the possibility of implementing selective breeding of bulls for better IVP performance.

Introduction
Genomic selection (GS) has revolutionized the cattle breeding industry through shortening generation interval, improving the accuracy of selection, reducing cost of breeding programs (Miglior et al., 2017). In this context, combining GS with Assisted Reproductive technologies (ARTs) such as multiple ovulation, embryo transfer and *in vitro* fertilization (IVF), can allow not only improving selection accuracy but also maximizing the intensity of selection (Ponsart et al., 2013; Kasinathan et al., 2015). To the extent that the use of artificial insemination (AI) has allowed rapid and wide-spread use of bulls of superior genetic merit, ARTs can increase the reproduction of superior females (Tonhati et al., 1999)

In vitro production of embryo (IVP) has become an alternative and highly competitive technique for multiple ovulation and embryo transfer (Boni, 2012), leading to increasing interest for harnessing the methodology, alongside GS, for rapid genetic gains in cattle and other livestock breeding. Several factors might affect the success of IVP in cattle including oocyte quality, sperm preparation methods, and media for *in vitro* maturation, fertilization and embryo culture (Akyol et al., 2014). Studies have additionally shown inter-bull differences in IVP performances (Eyestone and First, 1989; Hillery et al., 1990; Shi et al., 1990). However, to our knowledge, no study investigated the extent of genetic control of these differences between bulls in IVP performances. Therefore, the objective of this study was to estimate genetic parameters for bulls' contributions to IVP quality traits.

Material and Methods
IVP experiments.
IVP was performed in three different laboratories using the same chemically defined media from IVF Bioscience. All steps were performed according to manufacturer guidelines. In short: for each experiment, oocytes were aspirated from slaughterhouse ovaries, pooled and randomly divided

into two or three groups containing at least 50 oocytes. One of the two groups served as control and the same bull was used as a control for all IVP experiments. The other groups were used as test groups, where ejaculates from one of 123 test bulls were used for fertilization. Ejaculates from 123 bulls aged between 10- and 92 months at semen collection were provided by Viking Genetics (Randers, Denmark). For the statistical analysis, age of bulls was categorized into 3 classes: (1) peripubertal (less than 12 mo, mean age = 11 months), (2) pubertal (between 12 and 15 mo, mean age = 14 months), and (3) mature (more than 15 mo, mean age = 27 months) bulls. The following traits were assessed at day 7 and 8: i) Blastocyst rate (Blast) calculated as number of blastocysts over the total number of inseminated oocytes; ii) Hatch rate (Hatch) was defined as the proportion of blastocysts that are hatched. Kinetic obtained by visual classification of each blastocyst as non-expanded, expanded or hatching/hatched, and scored as 1, 2 or 3, respectively; Additionally, a Blastocyst rate x Kinetic Score x Morphology score (BLxMxK) (Avery and Greve, 2004) was calculated for each bull. For this calculation, morphology was first defined by scoring blastocysts as poor, good or excellent (scored as 1, 2 or 3). A total of 259 records where available for the analysis. All records for each trait and bull were subsequently normalized to the control bull as:

$$Normalized_{Trait_i} = \frac{Testbull_{trait_i}}{Controlbull_{trait_i}}, \quad (1)$$

Where $Testbull_{trait_i}$ is the record for each test bull in trait i while $Controlbull_{trait_i}$ is the corresponding record measured for the control bull from the same experiment and in the same lab as for the test bull.

All bulls in the IVP experiments were genotyped using the Illumina Infinium BovineSNP50 BeadChip (50k). A total of 42,363 SNPs were available after quality control for the analyses.

Statistical analysis.

The REML approach in DMU was used to estimate variance components and genetic parameters (Madsen and Jensen, 2013). The statistical model to describe observations of IVP traits is:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \boldsymbol{Z_1}\mathbf{g} + \boldsymbol{Z_2}\mathbf{c} + \mathbf{e}, \quad (2)$$

Where $\mathbf{y}$ is the vector of phenotypes, $\boldsymbol{\beta}$ is the vector of fixed effects, i.e., bull age class (1-3), semen type (conventional and sexed), as well as month and year of oocyte collection, $\mathbf{X}$ is the incidence matrix relating observations with fixed effects, $\mathbf{g}$ is the vector of random additive genetic effects of bulls, $\boldsymbol{Z_1}$ is the incidence matrix relating observations with random genetic effects, $\mathbf{c}$ is the vector of permanent environmental effects of bulls, $\boldsymbol{Z_2}$ is the incidence matrix relating observations with permanent environmental effects, and $\mathbf{e}$ is the vector of residual effects. The random effects are assumed to be normally distributed. Thus $\mathbf{g} \sim N(\mathbf{0}, \mathbf{G}\sigma_g^2)$, where $\mathbf{G}$ is a genomic relationship matrix and σ_g^2 is the additive genetic variance; $\mathbf{c} \sim N(\mathbf{0}, \mathbf{I}\sigma_c^2)$, where $\mathbf{I}$ is an identity matrix and σ_c^2 is the permanent environmental variance; and $\mathbf{e} \sim N(\mathbf{0}, \mathbf{D}\sigma_e^2)$, where $\mathbf{D}$ is a diagonal matrix with elements $d_{ij} = \sqrt{(N_{oocyte_{test}} * N_{oocyte_{control}})}$, standardized to have a mean of one, to account for heterogeneous residual variances due to differences in the number of oocytes used in each record in the test and control. Heritability (h^2), was defined as:

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_c^2 + \sigma_e^2}. \quad (3)$$

Repeatability (t) was defined as:

$$t = \frac{\sigma_g^2+\sigma_c^2}{\sigma_g^2+\sigma_c^2+\sigma_e^2}. \quad (4)$$

Results

IVP traits across bull age. Figure 1 presents box plots of phenotypic values for the studied IVP traits in the 3 age classes. Bulls at younger age had significantly higher performances in all the studied IVP traits, except hatch rate at day 7 (Hatch_D7), compared to bulls at later ages.

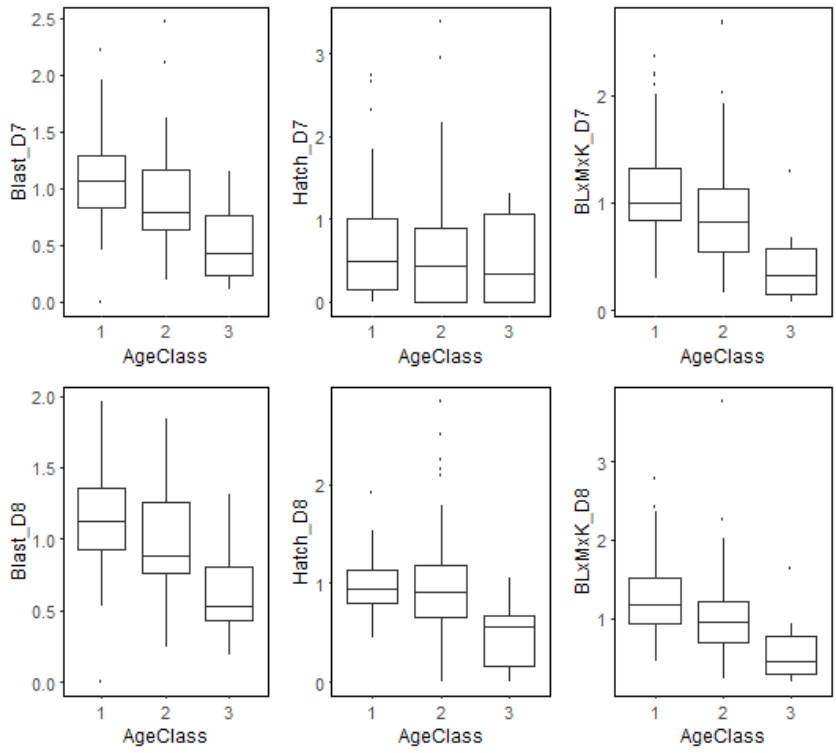

Figure 1. Plots of phenotypic measurements in IVP traits against age of bulls

Heritability and repeatability estimates. Estimates of phenotypic variance, heritability, and repeatability in the IVP traits are presented in Table 1. Generally, low to high heritability and moderate to high repeatability were estimated for the IVP traits. However, all estimates showed high standard errors. Compared to the IVP traits measured at day 7 after fertilization, relatively higher heritability and repeatability estimates were observed for the measurements on day 8.

Table 1. Phenotypic variance (σ_p^2), heritability (h^2, with standard errors) and repeatability (t, with standard errors) for IVP traits

Trait	σ_p^2	h^2(SE)	t (SE)
Blast_D7	0.14	0.08 (0.25)	0.46 (0.26)
Blast_D8	0.12	0.19 (0.27)	0.54 (0.27)
Hatch_D7	0.44	0.10 (0.32)	0.79 (0.33)
Hatch_D8	0.14	0.19 (0.21)	0.28 (0.22)

BLxMxK_D7	0.20	0.18e-5 (0.26)	0.55 (0.27)
BLxMxK_D8	0.24	0.46 (0.29)	0.61 (0.30)

Discussion

Blastocyst rates, morphology and kinetics have long been suggested as reliable indicators of the quality of an IVP system (Avery and Greve, 2004). Understanding the genetic underpinnings of these traits would allow implementation of selective breeding on bulls for enhanced performance in *in vitro* production of embryos. Despite high standard errors across estimates, our study suggests potentially substantial genetic control of the bull's contribution to the indicators of IVP success. Further studies with more sample size might be needed to validate the parameter estimates with probably lower standard errors. The study also reports significant effects of bull age on the IVP quality parameters. Effect of bull age have similarly been reported for male fertility related traits such as semen quality parameters (Olsen et al., 2020; Gebreyesus et al., 2021). Semen from younger bulls appear to result in higher blastocyst and hatch rates as well as BLxMxK score at day 7 and 8 after fertilization. These results can provide useful inputs towards planning successful IVP systems.

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