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Association of fertility traits with embryo development and pregnancy establishment

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ABSTRACT

Selection for fertility traits, such as daughter pregnancy rate (DPR), heifer conception rate (HCR), and cow conception rate, has been shown to improve pregnancy success within dairy herds. This study explored the relationship between fertility traits and embryo development in both in vitro and in vivo conditions in dairy cattle. Data from 2,408 in vitro–produced (IVP) and 1,801 in vivo–derived (IVD) embryo procedures were analyzed. Embryos produced in these procedures were classified by quality (grade 1, 2, and 3). We assessed the relationship between fertility traits (DPR and HCR) and various embryo development parameters, including the total number of structures collected, blastocyst rate, and embryo quality, using generalized linear models. The analysis focused on female Holstein donors aged 10 to 20 mo. Fertility traits did not show a significant association with in vitro embryo production. However, in vivo, higher fertility values for DPR and HCR were associated with improved blastocyst rates, suggesting that these fertility traits may have a greater influence on embryo development in vivo. In addition, higher HCR values in the embryo were associated with increased pregnancy per transfer by d 30 when embryos were transferred into both heifers and cows, resulting in more calves born. Higher DPR values were also associated with a higher proportion of calves born when embryos were transferred to cows, indicating the potential influence of DPR on pregnancy outcomes in lactating cows. Our findings show that fertility traits DPR and HCR are associated with improved embryo development and competence to establish pregnancy, highlighting the potential for selection for embryo developmental and reproductive success. Further research is needed to better understand the mechanisms underlying these effects and to identify genetic markers and individual genes that could enhance fertility outcomes.

Key words: fertility traits, embryo development, pregnancy success, embryo recipient

INTRODUCTION

Fertility plays a crucial role in the productivity and economic efficiency of dairy herds. In the United States, the main traits used to assess female fertility in dairy cattle are daughter pregnancy rate (**DPR**), cow conception rate (**CCR**), and heifer conception rate (**HCR**; VanRaden et al., 2004; Kuhn et al., 2006; Gobikrushanth et al., 2020). Daughter pregnancy rate quantifies how quickly lactating cows return to pregnancy, expressed as the percentage of open cows that become pregnant every 21 d (VanRaden et al., 2004), whereas HCR and CCR measure the ability of a heifer and a cow, respectively, to conceive following insemination (Kuhn et al., 2006; Gobikrushanth et al., 2020). Despite the low heritability of these traits (VanRaden et al., 2004, 2021), several studies have shown that animals with high genetic merit for fertility have improved reproductive performance (Ortega et al., 2017a; Lima et al., 2020; Sitko et al., 2023).

Measuring phenotypes associated with reproduction noninvasively and in a large number of animals is difficult and limits our ability to improve current fertility traits. However, a few studies have attempted to do so. For example, a study by Chebel and Veronese (2020) found that cows with high genetic merit for fertility had a shorter period to first estrus postpartum. The same group found that, in heifers, those with higher values for DPR exhibited larger follicular diameters and higher estradiol (**E_2**) concentrations compared with the low DPR group. However, this association was not observed with HCR

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-26. Nonstandard abbreviations are available in the Notes.

(Veronese et al., 2019). Although these traits have a genetic correlation of 0.514 (VanRaden et al., 2021), they may not explain the same phenotypes associated with fertility.

Single nucleotide polymorphisms in candidate genes associated with fertility traits have also been associated with embryo development in vitro and their adaptation to stressful environmental conditions (Cochran et al., 2013a,b; Ortega et al., 2016b, 2017b), lipid metabolism, and conceptus elongation (Abdollahi-Arpanahi et al., 2019). This underscores the importance of genetics in embryo development and pregnancy establishment, raising the question of whether embryos derived from cows with high genetic merit for fertility are more likely to produce viable embryos and achieve pregnancy.

The objectives of this study were to associate the fertility traits DPR and HCR with embryo development in both in vivo and in vitro conditions and to determine the association of fertility traits with the ability of an in vitro–produced (**IVP**) embryo to establish pregnancy. It was hypothesized that higher genetic merit for fertility is associated with improved embryo development to the blastocyst stage and that IVP embryos from high-fertility donors have higher pregnancy per embryo transfer.

MATERIALS AND METHODS

This was a retrospective study with embryo production data provided by Peak Genetics (URUS Group LP, Madison, WI). Two datasets were used, one that included embryo production from in vivo (in vivo–derived; **IVD**) and in vitro (IVP) procedures, and another one for transfers and pregnancy outcomes from IVP embryos.

Study 1. Association of Fertility Traits with Embryo Production In Vitro and In Vivo

Each observation corresponded to a collection for IVD or IVP and included: the dam (donor) ID, breed, birth date, fertility genetic values (DPR, HCR), date of procedure, location, and technician. Information regarding superstimulation and synchronization protocols used for in vivo embryo collection was not available and therefore could not be included as fixed effects in the statistical analyses. For IVP embryos, data included the total number of cumulus-oocyte complexes (**COC**) per aspiration procedure, blastocyst number, and their grade based on International Embryo Technology Society (**IETS**) guidelines (Barfield and Demetrio, 2022), where grade 1 embryos were considered the highest quality and grade 3 the lowest. For IVD embryos, data included structures recovered per flush procedure (unfertilized oocytes or uncleaved embryos, degenerated embryos, morulae, and blastocysts, per the IETS guidelines), the number of blastocysts, and their grade.

Data Filtering for Statistical Analysis. Analyses were performed on procedures from January 2020 to July 2024 in order to ensure that all data aligned with the most recent genetic base change available for the dataset (the 2020 genetic base), and only Holstein females ranging from 10 to 20 mo old were included. Pregnancy status of the heifer was not available and therefore could not be included in the analysis. To control for environmental conditions, season was added to the observations based on the month of occurrence (winter = December, January, February; spring = March, April, May; summer = June, July, August; fall = September, October, November), and blocks were created by combining the farm location and laboratory, the year, and the season of each procedure. Only blocks with at least 10 observations were considered for analysis. In vitro and in vivo embryo production were analyzed independently. In addition, a separate analysis was done to determine the differences in the response variables between IVD and IVP by filtering cows that have gone through both procedures at least once.

For the IVD analysis, a total of 617 donors with a total of 1,801 observations were used. The data included 5 farms and 5 laboratories in a total of 43 blocks. The IVP data analysis included 429 donors with a total of 2,408 observations. The data included 2 different laboratories and 5 different farm locations, in a total of 57 blocks. In addition, a group of 235 cows that had gone through both procedures was used to analyze the differences between IVD and IVP.

Statistical Analysis. All statistical analyses were conducted using R v4.4.0. The package lme4 was used to fit generalized linear mixed-effects models. The package emmeans was also used to estimate the LSM of the variables. Most of the analyses were computed using the following model:

$$y = \beta_0 + \beta_1 \times \text{BV} + \text{BLOCK} + \text{ID} + \text{e},$$

where the response variable, y, was modeled with β_0 representing the intercept and β_1 as the coefficient for the effect of the breeding value (BV) for either DPR or HCR. Fertility traits were analyzed separately due to multicollinearity, using a linear regression model. Random effects included BLOCK to account for environmental and management effects; donor ID, as there were multiple procedures per donor; and residual deviance (e). The responses (y) were the total number of structures collected, percentage of embryos produced, and percentage of grade 1, 2, and 3 embryos per procedure. To analyze the total number of structures, the lmer function in R was used with a square root transformation to adjust normality. For analyses of proportions or percentages, a glmer function was used employing a cbind function of successful versus nonsuccessful events. To estimate the proportion of embryos produced, a minimum of one structure recovered per procedure was required. To determine the proportion of grade 1, 2, and 3 embryos, at least one embryo was required per procedure.

To compare the differences between IVP and IVD, a similar model was used:

$$y = \beta_0 + \text{FTYPE} + \text{BLOCK} + \text{ID} + \text{e},$$

where y is the response variable, β_0 is the intercept, and fixed effects were the type of procedure implemented (FTYPE; IVP or IVD). Random effects included BLOCK, ID of the donor, and residual variance (e). Response variables were the number of structures collected, the number of blastocysts produced, blastocyst rate, and the percentage of grade 1, 2, and 3 embryos per procedure.

Study 2. Association of Fertility Traits with Pregnancy Outcomes Following Embryo Transfer

In this dataset, each observation was a transferred embryo and included information about the production technique (IVD or IVP), embryo type (fresh or frozen), embryo grade (1, 2, or 3), dam and sire ID and their genetic values for fertility, pregnancy status at d 30 and 60, and pregnancy outcomes (whether a live calf was born). In addition, data included recipient ID, breed, and classification (heifer or cow). Detailed information regarding estrus detection or synchronization protocols used for embryo transfer was not available in this dataset and could not be directly included in the statistical analyses.

Data Filtering and Selection Criteria. The embryos used in this analysis were derived from the previous dataset, with embryos produced from 2020 onward and transferred into recipients until September 2023, as those should have completed their gestation by July 2024. Only Holstein donors aged between 10 and 20 mo were included. Due to the limited number of IVD-transferred embryos included in the dataset (n = 36), only IVP embryos that were either grade 1 or grade 2 were used in the analysis. The season for each embryo transfer was determined as explained above. Data were organized into blocks based on farm location, technician who realized the transfer, year, and season of the transfer, with a minimum of 10 observations per block.

The first round of analyses evaluated embryo type and processing. The first analysis evaluated differences in pregnancy outcomes between grade 1 and 2 embryos that were either fresh or frozen, including a total of 10,676 transfers. The second analysis removed the effect of embryo grade and freezing and only compared pregnancy outcomes for transfers of fresh grade 1 embryos into heifers (n = 5,114) versus cows (n = 1,400). Lastly, follow-up analyses focused specifically on transfers involving fresh grade 1 embryos, with separate evaluations for transfers into heifers (n = 5,079) and cows (n = 1,356). Pregnancy outcomes are reported as pregnancy per embryo transfer (**P/ET**); for consistency with existing literature, the term pregnancy rate is used throughout the manuscript to denote P/ET.

To evaluate the effect of the genetic merit for fertility of the embryo on pregnancy outcomes, a parent average for HCR and DPR was calculated for each transferred embryo. Likewise, to determine the effect of recipient genetic merit on pregnancy outcomes, Holstein heifers with genetic values for fertility that received fresh grade 1 embryos were used (n = 69).

Statistical Analysis. Statistical analyses were performed using R v4.4.0, applying the lme4 package to fit generalized linear mixed-effects models, with the emmeans package used to estimate LSM for the variables. When significant main effects or interactions were detected, pairwise comparisons among LSM were performed using Tukey's honestly significant difference adjustment to control for multiple comparisons. The following model was used to evaluate the data from all embryo transfers:

$$y = \beta_0 + \text{EMBTYPE} + \text{EMBGR} + \text{EMBTYPE:EMBGR} + \text{RTYPE} + \text{BLOCK} + \text{DID} + \text{e},$$

where y is the response variable; β_0 is intercept; and fixed effects were the type of embryo (EMBTYPE), its grade (EMBGR), their interaction (EMBTYPE:EMBGR), and the type of recipient used in the transfer (RTYPE; heifer or cow). Random effects included BLOCK to account for environment and management during the transfer, and donor ID (DID), as there were multiple embryos derived per donor. Response variables were the pregnancy status of a transferred embryo, computed as binary data for success (1) or non-success (0) at d 30, 60, and if the pregnancy resulted in a calf. For pregnancy loss from d 30 to 60, only recipients pregnant at d 30 were considered, and for pregnancy loss after d 60, recipients pregnant at d 60 were considered for analysis. To evaluate the association between recipient type and pregnancy from fresh and grade 1 embryos, a similar model was used:

$$y = \beta_0 + \text{RTYPE} + \text{BLOCK} + \text{DID} + \text{e},$$

where y was the response variable and β_0 the intercept, the type of recipient used in the transfer was set as a fixed effect. Random effects included BLOCK and ID of the donor (DID). Response variables were pregnancy status at d 30, 60, if pregnancy ended in a calf, and pregnancy loss at d 60 and until calving.

A similar model was applied to evaluate the impact of fertility traits on pregnancy outcomes from fresh and grade 1 embryos:

$$y = \beta_0 + \beta_1 \times \mathrm{BV} + \mathrm{BLOCK} + \mathrm{DID} + \mathrm{e},$$

where the response variable y was modeled with β_0 as the intercept and β_1 as the coefficient for the effect of breeding value (BV), and BV represented the values of DPR or HCR from both the donor or the embryo. Random effects included BLOCK and donor ID (DID). For embryo fertility analysis, the sire's ID (SID) was also considered as a random effect. Response variables included pregnancy status at d 30 and 60, whether pregnancy resulted in a calf, and pregnancy loss at d 60 and until calving. Transfers into cows and heifers were analyzed separately.

To evaluate the fertility of the recipient on pregnancy outcome, the following model was used:

$$y = \beta_0 + \beta_1 \times \mathrm{BV_R} + \mathrm{BLOCK} + \mathrm{DID} + \mathrm{e},$$

where y was the response variable, β_0 the intercept, and β_1 the coefficient for the effect of the recipient's breeding value ($\mathrm{BV_R}$) on fertility based on HCR or DPR. Random effects included BLOCK and ID of the donor. Response variables were pregnancy status at d 30 and 60, if pregnancy ended in a calf, and pregnancy loss at d 60 and until calving.

RESULTS

Study 1. Embryo Production

Comparison of In Vitro and In Vivo Embryo Production Techniques. To compare in vitro and in vivo procedures, we evaluated females that underwent both procedures at least once. More oocytes were collected by follicular aspiration for in vitro embryo production ($P < 0.001$) than the number of structures recovered by flush for in vivo–derived embryos. The blastocyst rate (the number of blastocyst-stage embryos over either the number of structures flushed for IVP, or the number of COC collected and processed for IVP) was greater ($P < 0.001$) in IVD procedures compared with IVP, although the total number of blastocysts produced tended to be greater ($P = 0.06$) for IVP procedures. Embryo quality differed between procedures. There were fewer grade 1 embryos ($P < 0.001$) and more grade 2 and 3 ($P < 0.001$) embryos from IVP compared with IVD procedures (Table 1). Covariates included in the models, including donor identity and block effects, accounted for a portion of the variability in embryo production outcomes but did not alter the direction or significance of the associations between fertility traits and embryo development.

Impact of Donor Genetic Merit for Fertility on Embryo Production. We found no differences in the number of oocytes or structures recovered in IVP or IVD procedures based on DPR or HCR values of the donor ($P > 0.05$; Figure 1). Blastocyst rate in vitro was unaffected by fertility traits ($P > 0.05$; Figure 2A, B). However, in vivo, females with higher values for fertility traits had increased blastocyst rates ($P < 0.05$). It was estimated that for a unit increase in HCR the blastocyst rate increased by 2.97%, and each unit increase in DPR corresponded to a 1.88% increase in blastocyst rate (Figure 2C, D). Fertility traits did not have an overall effect on the proportion of grade 1 embryos produced in vivo ($P > 0.05$; Figure 3C, D). However, in vitro, females with higher values for

Table 1. Effect of type of procedure on embryo production; results presented as LSM ± SEM

Variable	In vitro embryo production[1]	In vivo embryo production[2]	P-value
Structures[3] (n)	13.5 ± 0.47^{a}	6.5 ± 0.39^{b}	<0.001
Blastocysts (n)	4.92 ± 0.22	4.31 ± 0.27	0.06
Blastocyst rate (%)	38.7 ± 1.26^{a}	57.3 ± 1.76^{b}	<0.001
Grade 1 embryos (%)	70.9 ± 1.23^{a}	84.5 ± 1.12^{b}	<0.001
Grade 2 embryos (%)	23.8 ± 2.38^{a}	15.4 ± 1.12^{b}	<0.001
Grade 3 embryos (%)	4.67 ± 0.48	—	

[a, b] Different superscripts within a row indicate differences ($P \leq 0.05$) between procedures.

[1] In vitro–produced: 1,220 procedures, 235 donors.

[2] In vivo–derived: 598 procedures, 235 donors.

[3] Structures: for in vitro procedures, this refers to COC collected. For in vivo procedures, this refers to all the structures collected from a flush, including unfertilized oocytes or uncleaved embryos, degenerated embryos, morulae, and blastocysts, following IETS guidelines.

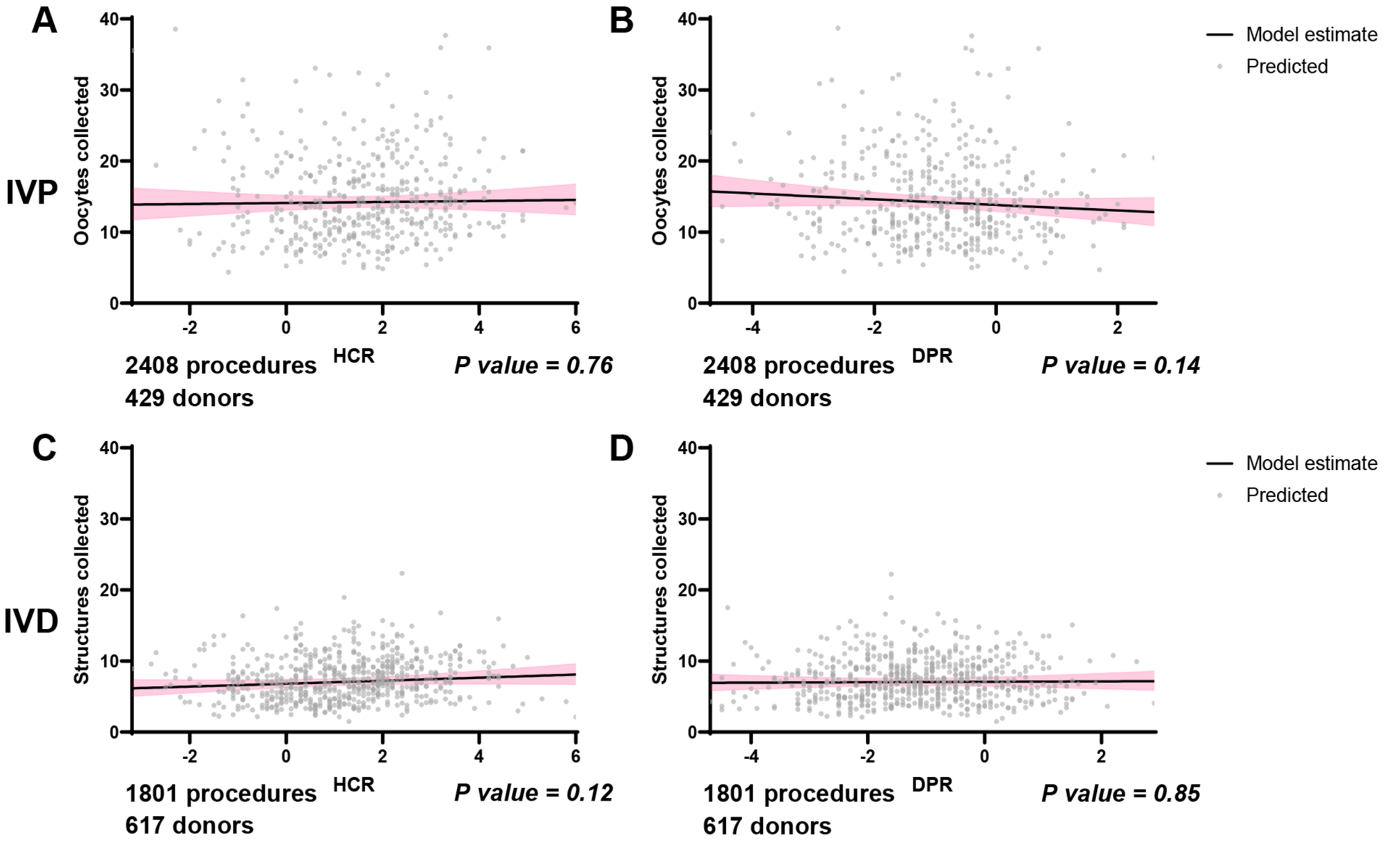

Figure 1. Effect of fertility traits on structures recovered per procedure. (A) Association between heifer conception rate (HCR) and oocytes recovered through ovum pick-up (OPU; $P = 0.76$). (B) Association between daughter pregnancy rate (DPR) and oocytes recovered through OPU ($P = 0.14$). (C) Association between HCR and structures recovered through uterine flush ($P = 0.12$). (D) Association between DPR and structures recovered through uterine flush ($P = 0.85$). Gray dots represent the predicted number of structures collected per donor. The 95% CI is presented in pink.

HCR tended ($P = 0.06$) to produce more grade 1 embryos (Figure 3A) and, consequently, fewer grade 2 embryos (Supplemental Figure S1A; see Notes). It was estimated that for each unit increase in HCR, grade 1 embryo rate increased by 0.52% (Figure 3A). This trend was not observed with DPR (Figure 3B; Supplemental Figure S1B).

Study 2. Pregnancy Outcomes of In Vitro–Produced Embryos

Influence of Embryo Type and Grade on Pregnancy Outcomes. Pregnancy outcomes from transferred embryos are presented in Table 2. To evaluate pregnancy per embryo transfer at d 30 and 60, as well as calf rate, a total of 10,676 embryo transfers derived from 352 donors were analyzed. For pregnancy loss between d 30 and 60, a total of 5,532 transfers that were pregnant at d 30 and originated from 322 donors were included. For pregnancy loss after d 60, a total of 5,017 transfers that remained pregnant at d 60 and originated from 322 donors were evaluated.

Fresh embryos exhibited a higher likelihood of achieving pregnancy by d 30 ($P < 0.001$) compared with frozen embryos (Table 2), and grade 2 embryos had a lower likelihood of pregnancy by d 30 compared with grade 1 embryos ($P < 0.001$). An interaction between embryo type and grade was also observed ($P < 0.05$), with no significant differences in pregnancy found between fresh and frozen grade 2 embryos, and both groups having lower pregnancy rates than fresh and frozen grade 1 embryos. This effect was maintained at d 60, and in the percentage of calves born ($P \leq 0.05$; Table 3). Pregnancy loss between d 30 and 60 was higher for grade 2 embryos compared with grade 1 embryos ($P = 0.003$). No significant effect was observed for pregnancy loss after d 60 for any embryo type ($P > 0.05$; Table 2).

Effect of Recipient Type on Pregnancy Outcomes. Table 3 summarizes pregnancy outcomes following embryo transfers into either cows or heifers. To evaluate all types of embryo transfers (grades 1 and 2, fresh and frozen), a total of 2,376 transfers into cows and 8,300 transfers into heifers were analyzed. In addition, pregnancy outcomes from fresh, grade 1 embryos were

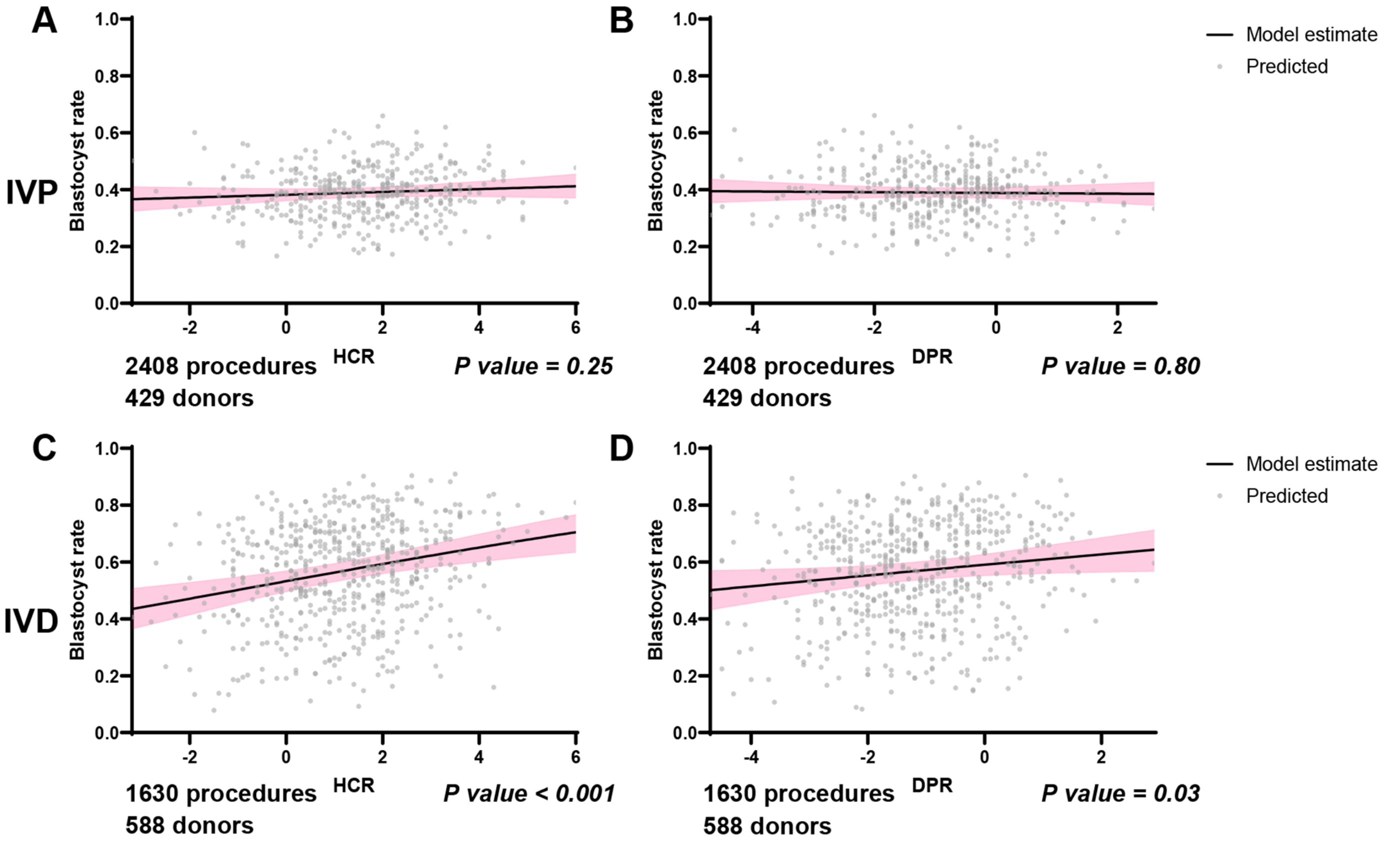

Figure 2. Effect of fertility traits on embryo production. (A) Association between HCR and blastocyst rate on in vitro procedures ($P = 0.25$). (B) Association between DPR and blastocyst rate on in vitro procedures ($P = 0.80$). (C) Association between HCR and blastocyst rate on in vivo procedures. For each increase in one HCR value, there is an estimated increase of 2.97% in blastocyst rate ($P < 0.001$). (D) Association between DPR and blastocyst rate on in vivo procedures. For each increase in one DPR value, there is an estimated increase of 1.88% in blastocyst rate ($P = 0.03$). Gray dots represent the predicted blastocyst rate per donor. The 95% CI is presented in pink.

evaluated, including 1,400 transfers into cows and 5,114 transfers into heifers. When evaluating all types of embryo transfers, pregnancy rate in heifer recipients was higher by d 30 ($P < 0.001$) and 60 ($P < 0.01$) compared with cows (Table 3). No differences in pregnancy loss between d 30 and d 60 were observed between heifers and cow recipients ($P > 0.05$). However, heifers experienced higher pregnancy loss after d 60 compared with cows for all types of embryo transfer, as well as when evaluating only transfers of fresh grade 1 embryos ($P < 0.05$). Despite this, heifers still produced more total calves compared with cows when all transfers are evaluated ($P < 0.001$; Table 3).

Influence of Donor Genetic Merit for Fertility on Pregnancy Outcomes. When evaluating pregnancy outcomes only from fresh embryos and quality grade 1 to heifer recipients, those derived from high-HCR females had increased pregnancy at d 30 ($P = 0.01$); it was estimated that each unit increase in HCR corresponded to a 1.32% rise in pregnancy rate by d 30. (Figure 4A). This

Table 2. Effect of the type and grade of the embryo on pregnancy success; results presented as LSM ± SEM

	Grade 1		Grade 2		*P*-value		
Variable	Fresh	Frozen	Fresh	Frozen	Embryo type	Embryo grade	Interaction
Pregnancy at day 30 (%)	58.1 ± 0.88^{a}	49.1 ± 1.58^{b}	36.7 ± 1.13^{c}	35.6 ± 3.58^{c}	<0.001	<0.001	0.06
Pregnancy at day 60 (%)	53.2 ± 0.91^{a}	44.1 ± 1.56^{b}	32.4 ± 1.09^{c}	32.6 ± 3.50^{c}	<0.001	<0.001	0.03
Calf rate (%)	49.0 ± 0.95^{a}	40.0 ± 1.57^{b}	29.7 ± 1.08^{c}	31.2 ± 3.48^{c}	<0.001	<0.001	0.01
Pregnancy loss d 30 to 60 (%)	7.70 ± 0.64^{a}	9.76 ± 1.31^{b}	10.57 ± 1.14^{b}	6.78 ± 2.98^{ab}	0.08	0.003	0.13
Pregnancy loss d 60 to parturition (%)	6.63 ± 0.65	8.40 ± 1.30	7.20 ± 0.98	4.23 ± 2.41	0.12	0.51	0.18

$^{a-c}$Different superscripts within a row indicate a difference (Tukey-adjusted comparisons; $P \leq 0.05$) between embryo classification.

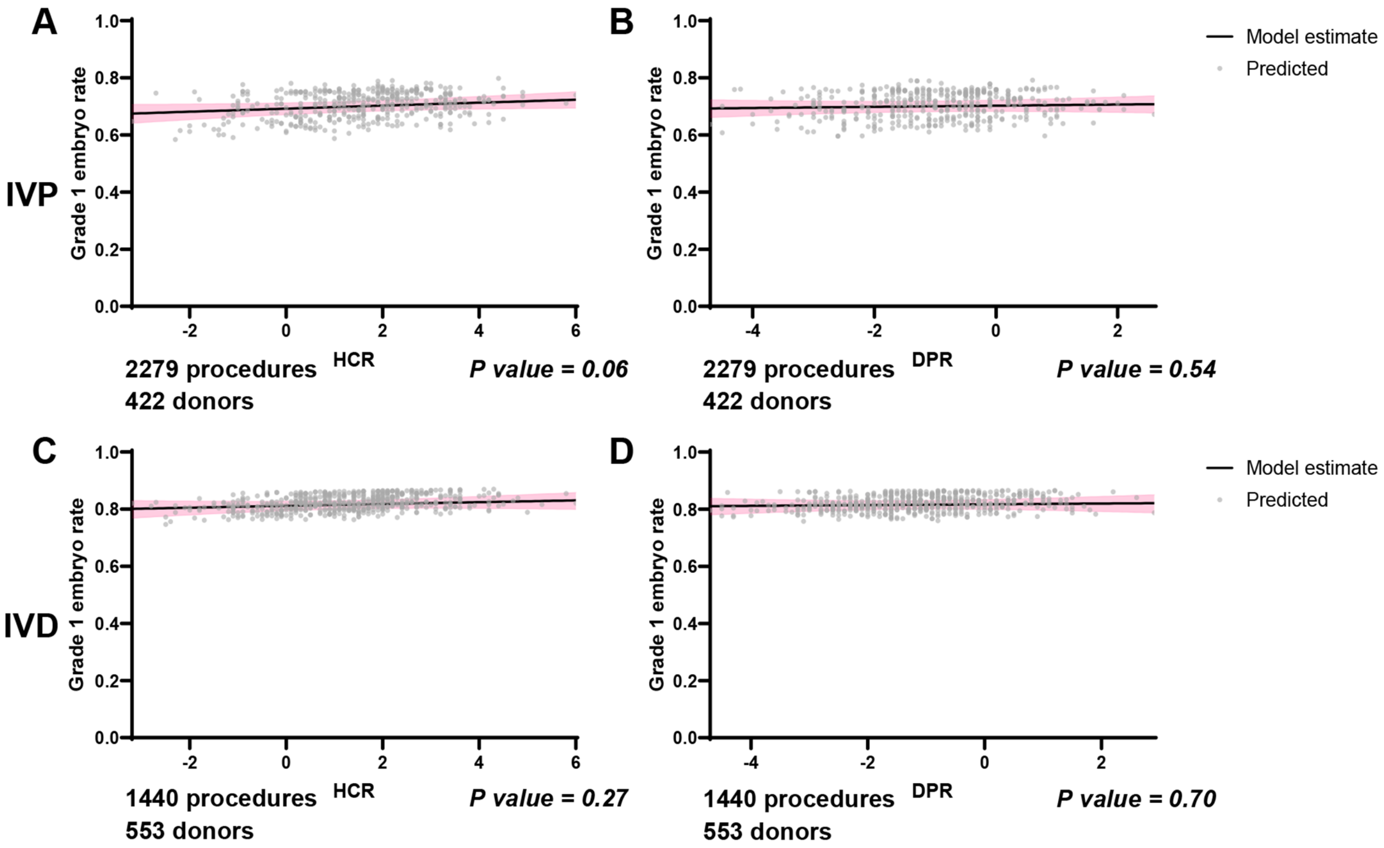

Figure 3. Effect of fertility traits on the proportion of grade 1 embryos produced. (A) Association between HCR and grade 1 embryo rate on in vitro procedures. For each increase in one HCR value, there is an estimated increase of 0.52% in proportion of grade 1 embryos produced (*P* = 0.06). (B) Association between DPR and grade 1 embryo rate on in vitro procedures (*P* = 0.54). (C) Association between HCR and grade 1 embryo rate on in vivo procedures (*P* = 0.27). (D) Association between DPR and grade 1 embryo rate on in vivo procedures (*P* = 0.70). Gray dots represent the predicted proportion of grade 1 embryos produced per donor. The 95% CI is presented in pink.

was not observed for pregnancy rate by d 60 ($P = 0.11$; Figure 4B). However, embryos derived from high-HCR donors tended ($P = 0.06$) to produce more calves, with an estimated increase of 1.07% in calf rate for each unit increase in HCR (Figure 4C). We found no effect of HCR on pregnancy loss from d 30 to 60 ($P = 0.10$), or after d 60 ($P = 0.45$; Supplemental Figure 2A, B; see Notes). In cow recipients, embryos coming from high-HCR donors had increased pregnancy rates by d 30, 60, and produced more calves ($P < 0.05$). For each unit increase in HCR, we observed were increases in pregnancy rate by d 30, 60, and calf rate by 2.80, 2.43, and 2.59%, respectively (Figure 4D–F).

Daughter pregnancy rate of donors did not affect pregnancy outcomes ($P > 0.05$; Figure 5A–C) or pregnancy loss when embryos were transferred to heifers (Supplemental Figure S2E, F). When embryos were transferred into cows, pregnancy rate by d 60 was higher when using embryos from high-DPR donors ($P = 0.04$; Figure 5E), and these embryos resulted in more calves ($P = 0.01$; Figure 5F), with estimated increases of 2.67 and 3.18% for each one-unit increase of DPR, respectively.

Table 3. Effect of the type of recipient on pregnancy outcomes; results presented as LSM ± SEM

	All transfers			Fresh grade 1 embryos		
Variable	Cow	Heifer	*P*-value	Cow	Heifer	*P*-value
Pregnancy at day 30 (%)	41.1 ± 1.51	48.4 ± 1.25	<0.001	55.3 ± 1.50	61.4 ± 0.90	<0.001
Pregnancy at day 60 (%)	37.3 ± 1.49	43.4 ± 1.25	<0.001	51.2 ± 1.48	56.0 ± 0.91	0.003
Calf rate (%)	35.0 ± 1.49	39.5 ± 1.26	<0.001	47.9 ± 1.56	50.9 ± 1.00	0.08
Pregnancy loss, d 30 to 60 (%)	8.41 ± 1.30	8.75 ± 1.13	0.74	7.18 ± 1.02	8.25 ± 0.67	0.35
Pregnancy loss, d 60 to parturition (%)	5.52 ± 1.10	7.48 ± 1.20	0.04	4.44 ± 0.88	7.70 ± 0.83	0.006

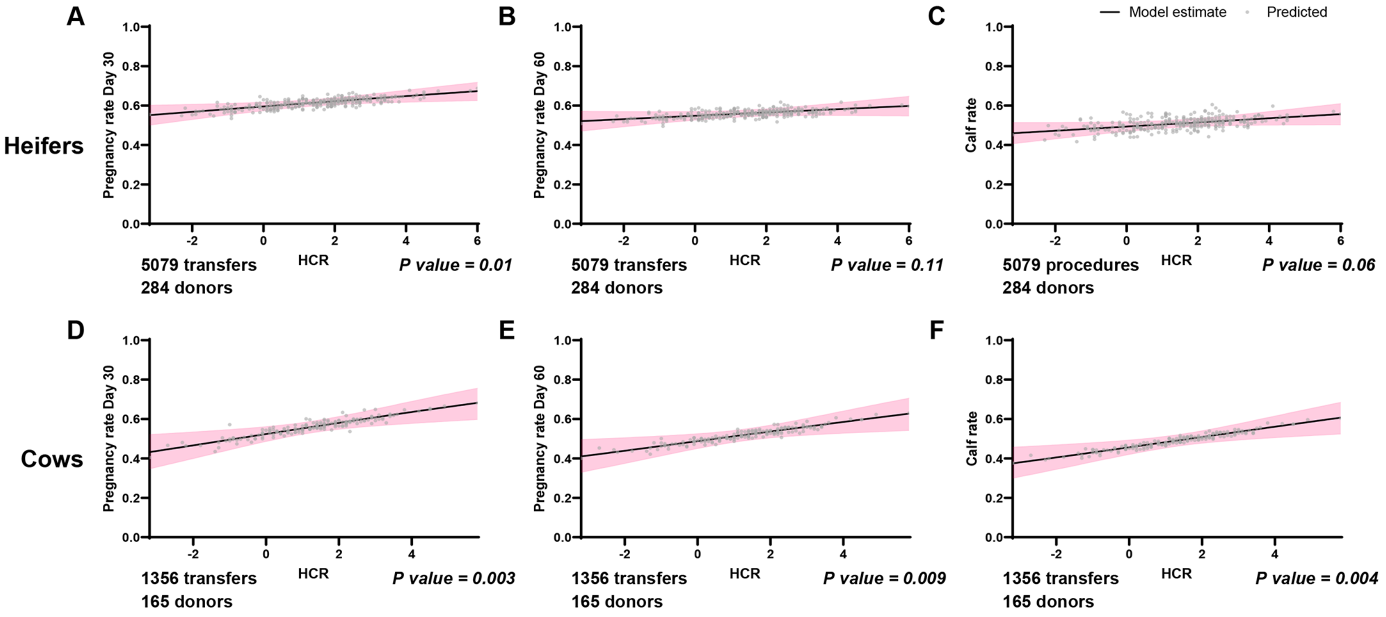

Figure 4. Effect of donor's HCR on pregnancy outcomes from fresh and grade 1 transferred embryos. (A) Association between HCR and pregnancy rate at d 30 on heifers. For each increase in one HCR value, there is an estimated increase in pregnancy rate by 1.32% ($P = 0.01$). (B) Association between HCR and pregnancy rate at d 60 on heifers ($P = 0.11$). (C) Association between HCR and proportion of calves born from heifers. For each increase in one HCR value, there is an estimated increase in calves born by 1.07% ($P = 0.06$). (D) Association between HCR and pregnancy rate at d 30 on cows. For each increase in one HCR value, there is an estimated increase in pregnancy rate by 2.80% ($P = 0.003$). (E) Association between HCR and pregnancy rate at d 60 on cows. For each increase in one HCR value, there is an estimated increase in pregnancy rate by 2.43% ($P = 0.009$). (F) Association between HCR and proportion of calves born from cows. For each increase in one HCR value, there is an estimated increase in calves born by 2.59% ($P = 0.004$). Gray dots represent the predicted pregnancy or calf rate per donor. The 95% CI is presented in pink.

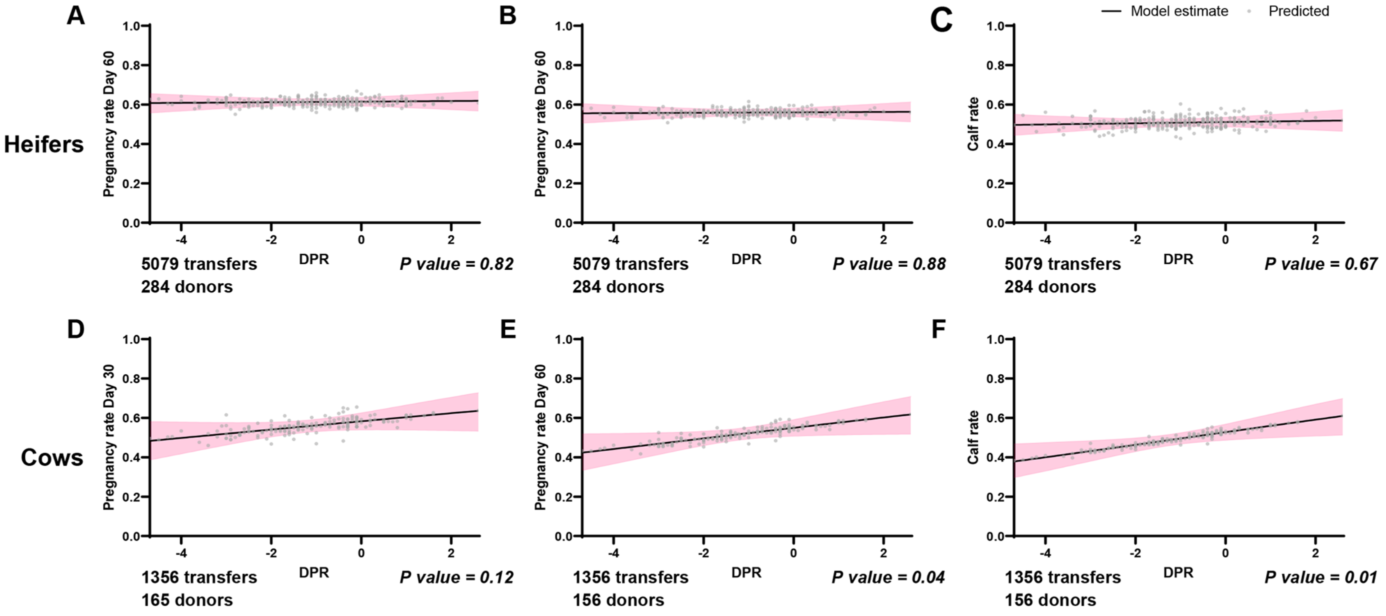

Figure 5. Effect of donor's DPR on pregnancy outcomes from fresh and grade 1 transferred embryos. (A) Association between DPR and pregnancy rate at d 30 on heifers ($P = 0.82$). (B) Association between DPR and pregnancy rate at d 60 on heifers ($P = 0.88$). (C) Association between DPR and proportion of calves born from heifers ($P = 0.67$). (D) Association between DPR and pregnancy rate at d 30 on cows. For each increase in one DPR value, there is an estimated increase in pregnancy rate by 2.09% ($P = 0.12$). (E) Association between DPR and pregnancy rate at d 60 on cows. For each increase in one DPR value, there is an estimated increase in pregnancy rate by 2.67% ($P = 0.04$). (F) Association between DPR and proportion of calves born from cows. For each increase in one DPR value, there is an estimated increase in calves born by 3.18% ($P = 0.01$). Gray dots represent the predicted pregnancy or calf rate per donor. The 95% CI is presented in pink.

Impact of Embryo Genetic Merit for Fertility on Pregnancy Success. Embryos with higher values for HCR based on their parent average were more likely to achieve pregnancy by d 30, regardless of whether they were transferred into heifers or cows ($P \leq 0.05$). It was estimated that for each unit increase of HCR, pregnancy rate at d 30 increased by 1.80% when transferred on heifers and 2.85% on cows (Figure 6A, D). Pregnancy loss from d 30 to 60 was greater ($P = 0.01$) for high-HCR embryos when transferred into heifers (Supplemental Figure S3A, see Notes). At the end of gestation, embryos with higher HCR tended to result in more calves, regardless of recipient type (heifers: $P = 0.09$; cows: $P = 0.07$). For each unit increase in HCR, it was estimated that the proportion of calves increased by 1.25% when transferred into heifers and 2.51% when transferred into cows (Figure 6C, F).

No association was found between the embryo's DPR and pregnancy outcomes when transferred into heifers ($P > 0.05$; Figure 7A–C). However, when embryos were transferred into cows, higher DPR values were associated with increased pregnancy rates by both d 30 and d 60 ($P < 0.05$), and resulted in more calves ($P = 0.01$). For each unit increase in DPR, there was an increase in pregnancy by d 30, 60, and proportion of calves by 3.71%, 4.53%, and 4.34%, respectively (Figure 7 D–F). Pregnancy loss tended to be lower for high-DPR embryos between d 30 and 60 ($P = 0.06$), but no differences were found in pregnancy loss after d 60 (Supplemental Figure S3G, H).

Recipient Genetic Merit for Fertility and its Effect on Pregnancy Outcomes. We found no effect of recipient HCR value on pregnancy outcomes at d 30 ($P = 0.88$), d 60 ($P = 0.95$), or in pregnancy loss between d 30 and d 60 ($P = 0.93$). Furthermore, no significant differences were found in pregnancy loss from d 60 to parturition ($P = 0.59$) or in the calving rate ($P = 0.70$; Supplemental Figures S4A–C and S5A, B; see Notes). The recipient's DPR was also not associated with any of these variables ($P > 0.05$; Supplemental Figures S4D–F and S5C, D).

DISCUSSION

This work aimed to determine the association of fertility traits with embryo production in vitro or in vivo. In this study, donors with higher values of HCR and DPR produced more embryos per IVD procedure than donors with lower values of HCR and DPR. Previous research has found genes associated with early embryonic development, including embryo compaction, trophectoderm, and inner cell mass formation (Cochran et al., 2013a;

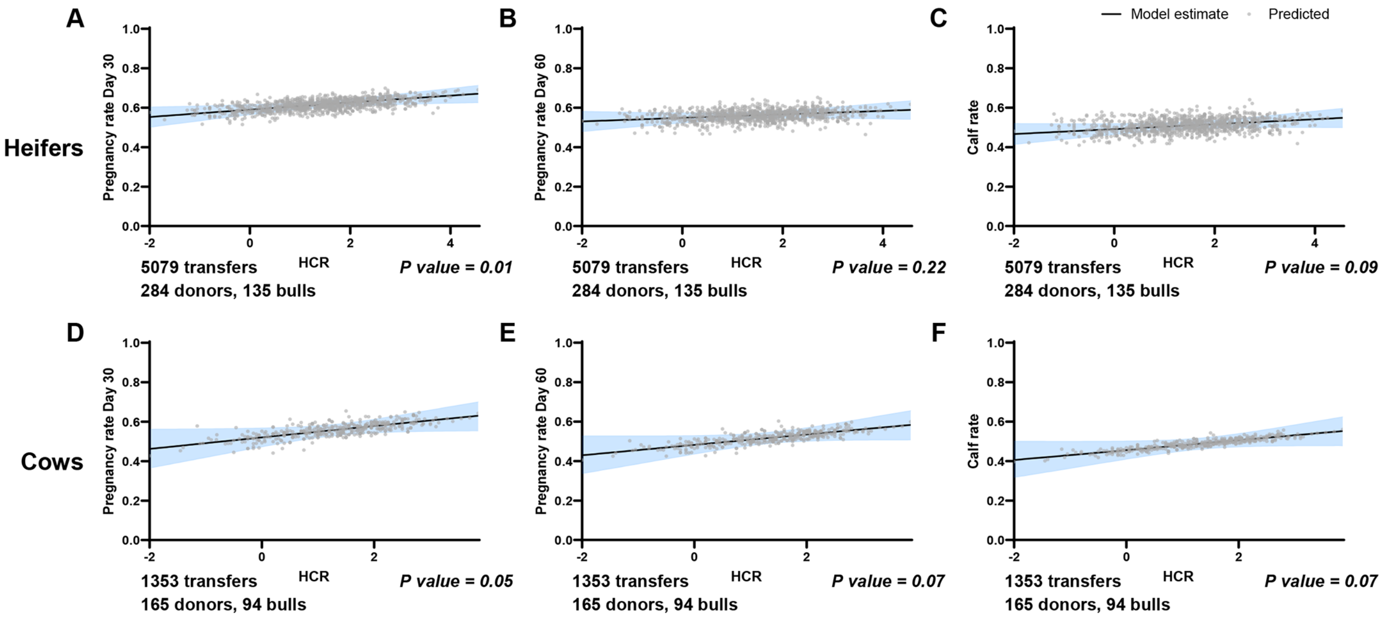

Figure 6. Effect of embryo's HCR on pregnancy outcomes from fresh and grade 1 transferred embryos. (A) Association between HCR and pregnancy rate at d 30 on heifers. For each increase in one HCR value, there is an estimated increase in pregnancy rate by 1.80% ($P = 0.01$). (B) Association between HCR and pregnancy rate at d 60 on heifers ($P = 0.22$). (C) Association between HCR and proportion of calves born from heifers. For each increase in one HCR value, there is an estimated increase in calves born by 1.25% ($P = 0.09$). (D) Association between HCR and pregnancy rate at d 30 on cows. For each increase in one HCR value, there is an estimated increase in pregnancy rate by 2.85% ($P = 0.05$). (E) Association between HCR and pregnancy rate at d 60 on cows. For each increase in one HCR value, there is an estimated increase in pregnancy rate by 2.63% ($P = 0.07$). (F) Association between HCR and proportion of calves born from cows. For each increase in one HCR value, there is an estimated increase in calves born by 2.51% ($P = 0.07$). Gray dots represent the predicted pregnancy or calf rate per combination between donor and sire. The 95% CI is presented in blue.

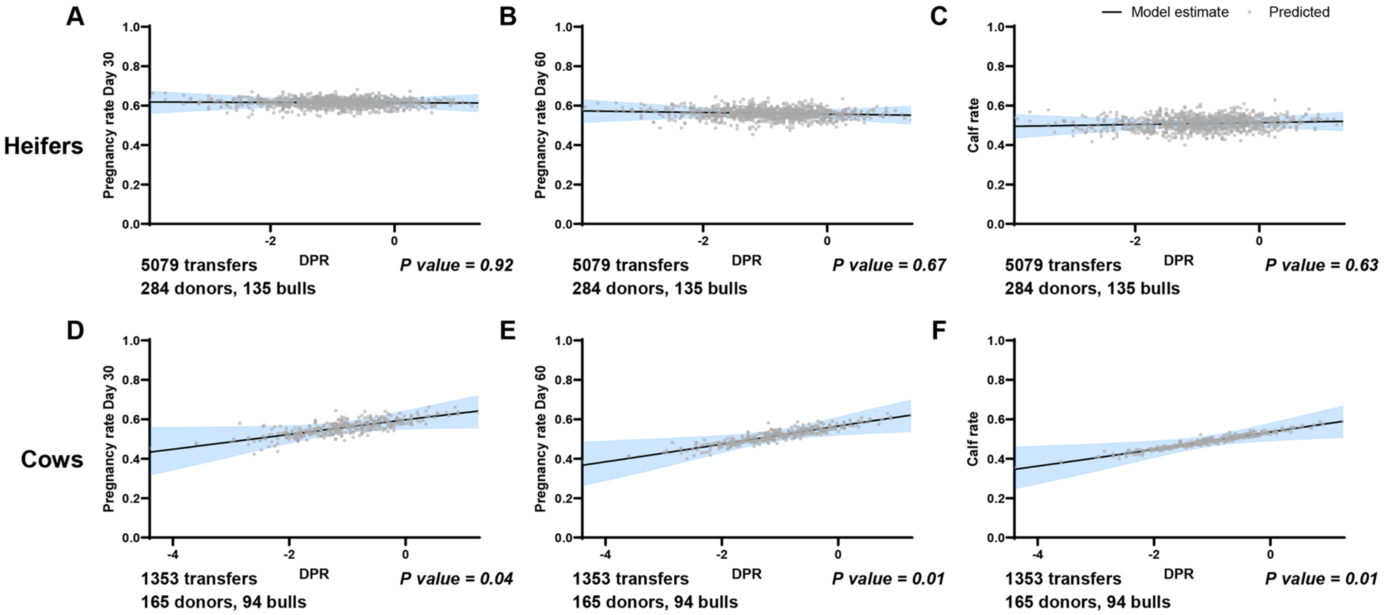

Figure 7. Effect of embryo's DPR on pregnancy outcomes from fresh and grade 1 transferred embryos. (A) Association between DPR and pregnancy rate at d 30 on heifers ($P = 0.92$). (B) Association between DPR and pregnancy rate at d 60 on heifers ($P = 0.67$). (C) Association between DPR and proportion of calves born from heifers ($P = 0.63$). (D) Association between DPR and pregnancy rate at d 30 on cows. For each increase in one DPR value, there is an estimated increase in pregnancy rate by 3.71% ($P = 0.04$). (E) Association between DPR and pregnancy rate at d 60 on cows. For each increase in one DPR value, there is an estimated increase in pregnancy rate by 4.53% ($P = 0.01$). (F) Association between DPR and proportion of calves born from cows. For each increase in one DPR value, there is an estimated increase in calves born by 4.34% ($P = 0.01$). Gray dots represent the predicted pregnancy or calf rate per combination between donor and sire. The 95% CI is presented in blue.

Ortega et al., 2016a, 2017a). Downregulation or inhibition of those genes has been shown to impair embryo development and even cause embryonic death (Thiel et al., 2006; Rantakari et al., 2010; Chan et al., 2016; Ortega et al., 2017b), suggesting, as do the results of this study, that some of the genes which explain fertility traits are associated with proper embryo development during the first week of gestation, increasing the chances to establish a pregnancy. To provide context for the population evaluated, the fertility genetic merit of animals included in this study differed from contemporary US Holstein averages reported by the Council on Dairy Cattle Breeding (**CDCB**); however, direct comparisons should be interpreted cautiously, as CDCB summaries primarily reflect cow populations, whereas donor animals in this study were predominantly heifers selected within genetic development programs.

In this study, associations between HCR and blastocyst rate were stronger than those observed for DPR. It is possible that HCR is more explanatory of embryo development than DPR. Even with a high genetic correlation between these traits (VanRaden et al., 2021), they may still reflect different aspects of fertility. This has been previously illustrated in a study with Holstein females genotyped as heifers and followed until after first calving. When evaluated by DPR, those with higher values had larger follicle diameters and higher E_2 concentrations as heifers. However, their HCR did not influence any of these measurements (Veronese et al., 2019; Chebel and Veronese, 2020).

In this study, fertility traits were not associated with in vitro embryo production. This is not surprising, as IVP systems impose a uniform developmental constraint that can reduce the expression of donor-specific biological advantages, rather than amplifying genetic differences among donors. Indeed, oviductal embryokines such as IGF1, CSF2, LIF, and FGF2 have been shown to regulate cell signaling pathways that affect early embryo development and their competence in vitro (Loureiro et al., 2011; Denicol et al., 2014; Hansen and Tríbulo, 2019; Tríbulo et al., 2019; Stoecklein et al., 2021; McDonald et al., 2025). Hence, under in vivo conditions, the fact that high-fertility females produce more embryos is an indicator of the genetic influence on their ability to provide a favorable environment for early embryo development. When embryos are produced in vitro, this maternal advantage is removed, masking developmental differences between high- and low-fertility donors. In beef cattle, for example, the uterine lumen of females classified as high-fertility had increased concentrations of prostaglandins, metabolites related to energy and amino acid metabolism, and increased expression in the uterus of genes related to transport of amino acids and growth factors (Moraes et al., 2018, 2020a,b). However, further research is required to determine if these differences exist in Holsteins classified by current fertility traits.

As previously shown in other studies (Hasler et al., 1995; Chebel et al., 2008; Demetrio et al., 2020), embryos with quality grade 1 had greater pregnancy rates compared with grade 2 embryos. High-HCR females also tended to produce more grade 1 embryos through IVP procedures, indicating that even in suboptimal in vitro conditions, high-fertility females produce embryos with more competency to establish pregnancy, making HCR a potential trait to select donors for embryo production. This is further supported by previous studies, where, when controlling only for sire genetics, markers associated with embryo cleavage and blastocyst production in vitro were identified, with some of these previously associated with fertility traits (Cochran et al., 2013b; Davenport et al., 2025).

The heritability of the number of viable embryos produced through in vitro procedures has been estimated to range from 0.01 to 0.187, whereas for in vivo embryo production, it ranged from 0.136 and 0.210 (Jaton et al., 2016; Parker Gaddis et al., 2017). This suggests that it is possible to select for increased embryo production, offering a potential method to improve outcomes in this technique. However, further research is required to identify genetic markers and candidate genes associated with embryo development and competence and fertility traits, controlling for the embryo genetics.

Another novel finding of this study is that embryos produced in vitro, coming from high-fertility females, had increased pregnancy rates when transferred to nonfertility classified recipients. This indicates that the genetics of the embryo do have an impact on its ability to establish pregnancy. It is possible that the embryo's genetic composition could affect its ability to interact with the maternal environment. Several studies have identified differentially expressed genes in blastocyst-stage embryos that are associated with increased pregnancy outcomes (Zolini et al., 2020a,b). Furthermore, a study from Ribeiro et al. (2016) found that in differentially expressed genes related to conceptus elongation, 39 SNP were associated with at least one fertility trait (DPR, CCR, or HCR). From these genes, 3 SNP were validated for their association with the reproductive performance of heifers, and 4 SNP with reproductive performance in cows (Abdollahi-Arpanahi et al., 2019).

The differences in DPR and HCR phenotypes on pregnancy outcomes may be due to distinct maternal environments between heifers and cows. Heifers and cows have different physiological conditions, particularly regarding lactation (Wiltbank et al., 2006), which could influence the way the embryo interacts with the maternal environment. Heifer conception rate seems to be more closely associated with genes related to embryonic competence, potentially enhancing the embryo's ability to establish pregnancy. This may explain why HCR is linked to better pregnancy outcomes in both heifers and cows. On the other hand, DPR may be more closely related to genes involved in communication between the maternal environment and the embryo, particularly in lactating dairy cows. This could explain why the effect of DPR is more pronounced in cows, which must establish pregnancy while simultaneously undergoing lactation, a process that does not happen in heifers and that could affect the uterine environment due to metabolic and hormonal changes. Several studies have shown that different fertility traits are associated with different chromosomal regions (Parker Gaddis et al., 2016), and that some genes may be associated with one trait but not with others (Cochran et al., 2013a; Ortega et al., 2016a), supporting the idea that each trait reflects different biological processes. Overall, these genetic influences highlight the complex interplay between the embryo and the maternal environment in determining pregnancy success, but further research is necessary to dissect these relationships.

A limitation of this retrospective study is the lack of detailed information regarding superstimulation, estrus detection, and synchronization protocols. Although these factors could not be explicitly modeled, the inclusion of block effects capturing farm location, laboratory, technician, season, and year likely mitigated a portion of the unmeasured variation associated with reproductive management.

No association was found between the genetic fertility trait of the recipient (recipient HCR) and pregnancy outcome. This is not surprising for HCR, as it appears to be more related to events leading up to proper early embryo development, rather than to pregnancy stages occurring after d 7 of insemination (time of blastocyst formation). It is possible that these results did not achieve significance due to the small number of transfers evaluated (n = 236). Further research is needed to confirm whether fertility traits play a role in enhancing the maternal environment and to identify specific candidate variants that could explain these phenotypes and, in turn, improve fertility traits. Genetic fertility evaluations analogous to DPR or HCR are not currently available for sires; therefore, phenotypic measures such as sire conception rate, which have not been shown to relate to in vitro embryo production (Ortega et al., 2018; Lockhart et al., 2023), were not considered in this study.

In conclusion, this study suggests that fertility traits such as HCR and DPR are associated with embryo development, but the effect varies between in vitro and in vivo conditions. Higher HCR and DPR values were linked to improved blastocyst rates in vivo, but not in vitro, likely due to differences in the environment where embryo development occurs. Furthermore, embryos with high genetic merit for fertility were associated with increased pregnancy outcomes. These findings underline the complex relationship between genetic traits, embryo development, and the maternal environment, but also provide insights into the potential for selection of donors with higher fertility values to achieve more pregnancies, ultimately leading to more calves. Further research is needed to explore specific genetic markers for each one of these phenotypes that could potentially enhance fertility outcomes.

NOTES

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Nonstandard abbreviations used: CDCB = Council on Dairy Cattle Breeding; COC = cumulus-oocyte complex; DPR = daughter pregnancy rate; E_2 = estradiol; HCR = heifer conception rate; IETS = International Embryo Technology Society; IVD = in vivo–derived; IVP = in vitro–produced; OPU = ovum pick-up; P/ET = pregnancy per embryo transfer.

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