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Sire contribution to fertilization failure and early embryo survival in cattle

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ABSTRACT

Despite passing routine laboratory tests of semen quality, bulls used in artificial insemination (AI) exhibit a significant range in field fertility. The objective of this study was to determine whether subfertility in AI bulls is due to issues of sperm transport to the site of fertilization, fertilization failure, or failure of early embryo or conceptus development. In experiment 1, Holstein-Friesian bulls (3 high fertility, HF, and 3 low fertility, LF) were selected from the national population of AI bulls based on adjusted fertility scores from a minimum of 500 inseminations (HF: +4.37% and LF: −12.7%; mean = 0%). Superovulated beef heifers were blocked based on estimated number of follicles at the time of AI and inseminated with semen from HF or LF bulls (n = 3–4 heifers per bull; total 19 heifers). Following slaughter 7 d later, the number of corpora lutea was counted and the uteri were flushed. Recovered structures (oocytes/embryos) were classified according to developmental stage and stained with 4′,6-diamidino-2-phenylindole to assess number of cells and accessory sperm. Overall recovery rate (total structures recovered/total corpora lutea) was 52.6% and was not different between groups. Mean (± standard error of the mean) number of embryos recovered per recipient was 8.7 ± 5.2 and 9.4 ± 5.5 for HF and LF, respectively. Overall fertilization rate of recovered structures was not different between groups. However, more embryos were at advanced stages of development (all blastocyst stages combined), reflected in a greater mean embryo cell number on d 7 for HF versus LF bulls. Number of accessory sperm was greater for embryos derived from HF than for LF bulls. The aim of experiment 2 was to evaluate the effect of sire fertility on survival of bovine embryos to d 15. Day 7 blastocysts were produced in vitro using semen from the same HF (n = 3) and LF (n = 3) bulls and transferred in groups of 5–10 to synchronized heifers (n = 7 heifers per bull; total 42 heifers). Conceptus recovery rate on d 15 was higher in HF (59.4%,) versus LF (45.0%). Mean length of recovered conceptuses for HF bulls was not affected by fertility status. In conclusion, while differences in field fertility among AI sires used in this study were not reflected in fertilization rate, differences in embryo quality were apparent as early as d 7. These differences likely contributed to the higher proportion of conceptuses surviving to d 15 in HF bulls.
Key words: bull fertility, embryo mortality, superovulation, conceptus elongation, spermatozoa

INTRODUCTION

Reproductive efficiency is a major driver of profitability in livestock production systems, particularly in seasonal systems (Shalloo et al., 2014). The majority of research effort in the field of bovine reproductive physiology has focused on understanding and improving cow fertility. This reflects the fact that gestation and lactation put significant metabolic stress on the cow that can have consequences for both oocyte and embryo quality as well as the reproductive tract environment (Walsh et al., 2011). The fact that, by definition, herd fertility tends to reflect female rather than male traits (e.g., postpartum resumption of cyclicity, submission rate, pregnancy per AI, calving interval, and so on) has resulted in much less emphasis being placed on male fertility and as a consequence the influence of the bull on herd fertility in both dairy and beef herds has been somewhat overlooked (Diskin et al., 2018). However, bull fertility can seriously affect overall reproductive performance, especially in individual herds if bulls with poor fertility are widely used. While the exact proportion of poor herd reproductive efficiency that can be explained by the fertility of an individual bull is difficult to establish, given that semen from elite bulls can be used simultaneously in several countries, often reaching >100,000 inseminations per year, the fertil-

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ity of an individual bull can have a major impact on productivity and economic returns.

The ability to predict a bull's fertility before semen is released into the field has been a long-term objective of the animal breeding industry; the recent shift in the dairy industry toward the intensive use of young genomically selected bulls has amplified this need as there is limited time for test inseminations (Taylor et al., 2018). Despite animal breeding centers employing stringent pre- and postthawed quality control checks, significant variation still exists in bull field fertility (Fair and Lonergan, 2018). Furthermore, while routine laboratory tests assess sperm function in terms of motility and morphology, they do not differentiate between sperm transport through the female reproductive tract, sperm fertilizing ability or ability to sustain early embryo development. Other functional laboratory assays such as sperm binding to oviductal epithelial cells and in vitro fertilization (**IVF**) test sperm ability to bind and fertilize an oocyte; however, by their nature, they do not assess the ability of sperm to get from the site of semen deposition to the site of fertilization and, in general, they poorly correlate with in vivo fertility (Fair and Lonergan, 2018).

Apart from traditional assessments of morphology and motility, other more objective tools including computer-assisted sperm analysis and flow-cytometry can aid in the decision to release for sale or discard a product (Harstine et al., 2018). While no single in vitro bioassay of sperm quality has been successful in predicting sire fertility, positive correlations have been reported when a multivariate approach has been taken (Sellem et al., 2015). Understanding the underlying causes of variation in bull fertility is a key prerequisite to achieving the goal of developing such a sperm bioassay (Fair and Lonergan, 2018) and must be coupled with knowledge of the physiological characteristics associated with semen processing as well as bull behavior and libido and proper management during semen collection to maximize output per bull (Schenk, 2018).

While there is a significant range in field fertility among bulls used in AI, it is not clear where along the developmental axis such differences in fertility originate. Although fertilization success is typically high (>85%) following AI in cattle, many of the resulting embryos fail to develop to term. A significant proportion of this loss occurs between fertilization and maternal recognition of pregnancy (Diskin and Morris, 2008; Berg et al., 2010; Pohler et al., 2020) which in cattle occurs around d 16 postfertilization; indeed, in high-producing dairy cows as many as 50% of embryos may no longer be viable by d 7 (Sartori et al., 2010; Wiltbank et al., 2016). A further proportion of loss occurs in the period of conceptus elongation and interferon-tau production associated with signaling to the uterus leading to failure of maternal recognition of pregnancy (Wiltbank et al., 2016; Forde and Lonergan, 2017).

Therefore, using 2 contrasting models (involving superovulation and multiple embryo transfer), the objective of the present study was to characterize the sire contribution to sperm transport, fertilization failure, and early embryo development, as well as conceptus growth and survival. We hypothesized that sperm derived from sires of divergent field fertility would exhibit differences in one or more of sperm transport, fertilization rate, or early embryo development and survival. Although others have recently addressed the contribution of the bull to fertility (Franco et al., 2018, 2020), they did not study very early stages of embryo development (pre- and peri-elongation period) and did not use direct methods to evaluate early embryonic losses (i.e., early embryo recovery and assessment).

MATERIALS AND METHODS

All experimental procedures involving animals were sanctioned by the Animal Research Ethics Committee of University College Dublin and were licensed by the Health Products Regulatory Authority, Ireland, in accordance with Statutory Instrument No. 543 of 2012 120 under Directive 2010/63/EU on the Protection of Animals Used for Scientific Purposes (http://www.irishstatutebook.ie/eli/2012/si/543/made/en/pdf).

Bulls

A panel of Holstein-Friesian bulls (n = 6) was selected for this study, from which cryopreserved semen was used commercially for AI in Ireland. Data on field fertility were obtained from the Irish Cattle Breeding Federation database based on an adjusted sire fertility index (Berry et al., 2011). Sire fertility was defined as pregnancy to a given service identified retrospectively either from a calving event or where a repeat service (or a pregnancy scan) deemed the animal not to be pregnant. These raw data were then adjusted for factors including semen type (frozen, fresh), cow parity, month of service, day of the week when serviced, service number, cow genotype, herd, AI technician, and bull breed. The adjusted sire fertility index given for each bull was then weighted for the number of service records, resulting in an adjusted pregnancy rate (Figure 1; mean = 0%). Holstein-Friesian bulls that had a minimum of 500 inseminations formed the base population (840 bulls; Figure 1), from which high fertility (HF, n = 3; +4.37%) and low fertility (LF, n = 3; −12.7%) bulls were selected with an average difference based on adjusted fertility scores of 17.1% (Figure 1).

Experiment 1. Effect of Sire Fertility Status on Fertilization Rate and Early Embryo Development

The aim of this experiment was to determine whether subfertility in AI bulls is due to issues of sperm transport to the site of fertilization (assessed based on accessory sperm number; i.e., sperm bound to the zona pellucida), fertilization failure, or compromised early embryo development. Heifers were housed indoors on slats for the duration of the experiment and were fed a diet consisting of grass and maize silage supplemented with a commercial beef ration. Superovulation was used to maximize the number of measures per heifer. Semen from HF (n = 3) and LF (n = 3) bulls was used to inseminate superovulated heifers (3–4 heifers per bull). The estrous cycles of Charolais- or Limousin-cross heifers (n = 19, 23.1 mo ± 4.7, 588.7 kg ± 41.9) were synchronized using an 8-d progesterone releasing intravaginal device (PRID E, 1.55 g of progesterone, Ceva Santé Animale). On the day of the PRID E insertion each heifer received a 2-mL i.m. injection of synthetic GnRH (Ovarelin, Ceva Santé Animale, equivalent to 100 μg of Gonadorelin). One day before PRID E removal all heifers were administered 5 mL of $PGF_{2\alpha}$ (Enzaprost, Ceva Santé Animale, equivalent to 25 mg of Dinoprost) intramuscularly. Ten days after standing estrus, heifers underwent a superstimulation treatment. Decreasing doses of follicle-stimulating hormone (**FSH**) were administered twice daily for 4 d (455 IU of FSH in total: Folltropin; Vetoquinol) together with $PGF_{2\alpha}$ (5 mL of Enzaprost) administered twice on the third day of FSH treatment (with the fifth and sixth FSH injection), and followed by AI with frozen-thawed semen 24 (d −1) and 36 h after the last FSH injection (Figure 2). Day 0 was considered as the day when fertilization occurred (28–32 h after observation of first standing estrus event; Randi et al., 2018). Heifers were blocked based on estimated number of follicles at the time of AI using transrectal ultrasonography and then inseminated with semen from HF or LF bulls (3–4 heifers per bull; 9–10 heifers per treatment). Heifers were slaughtered 7 d later, and their reproductive tracts were recovered following which the number of corpora lutea (**CL**) were counted and the uterus of each heifer was flushed with PBS. Re-

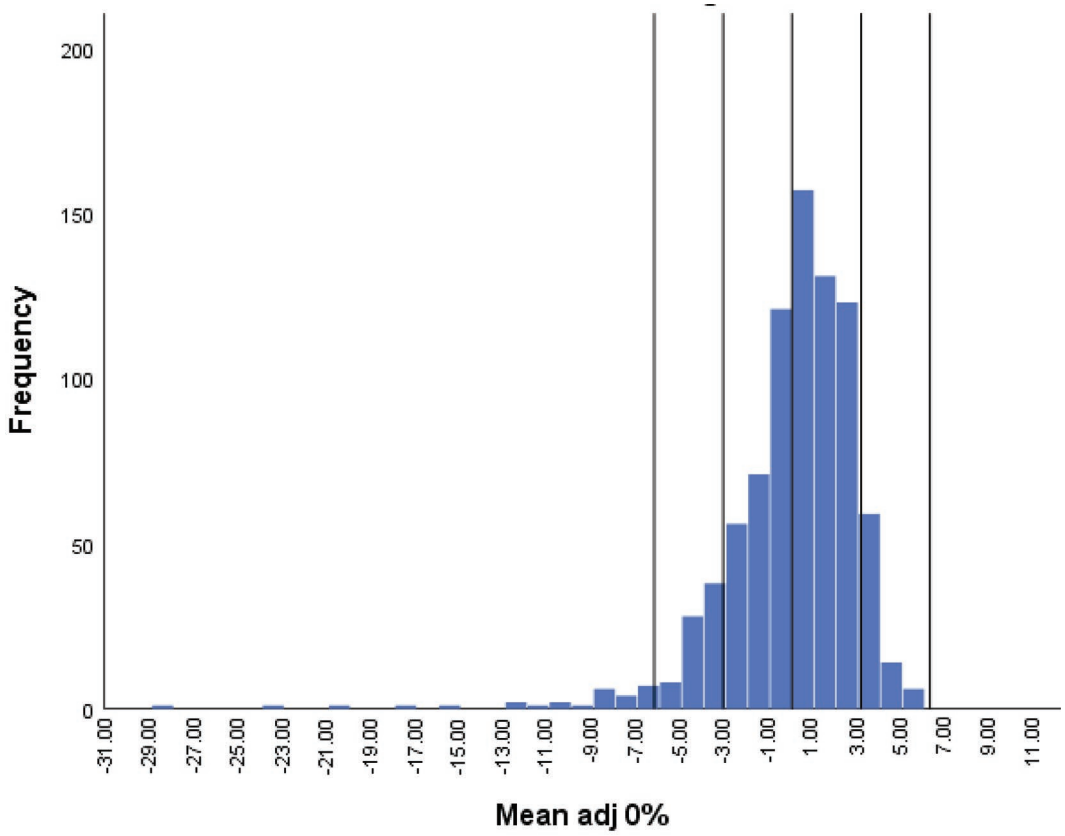

Figure 1. Mean adjusted (adj) fertility of Holstein-Friesian bulls that had a minimum of 500 inseminations forming the base population (840 bulls) from which high fertility (n = 3) and low fertility (n = 3) were selected with an average difference based on adjusted fertility scores of 17.1% between the 2 groups. Vertical lines indicate 1 and 2 SD (SD = 3.12) above and below the mean of the population (mean = 0).

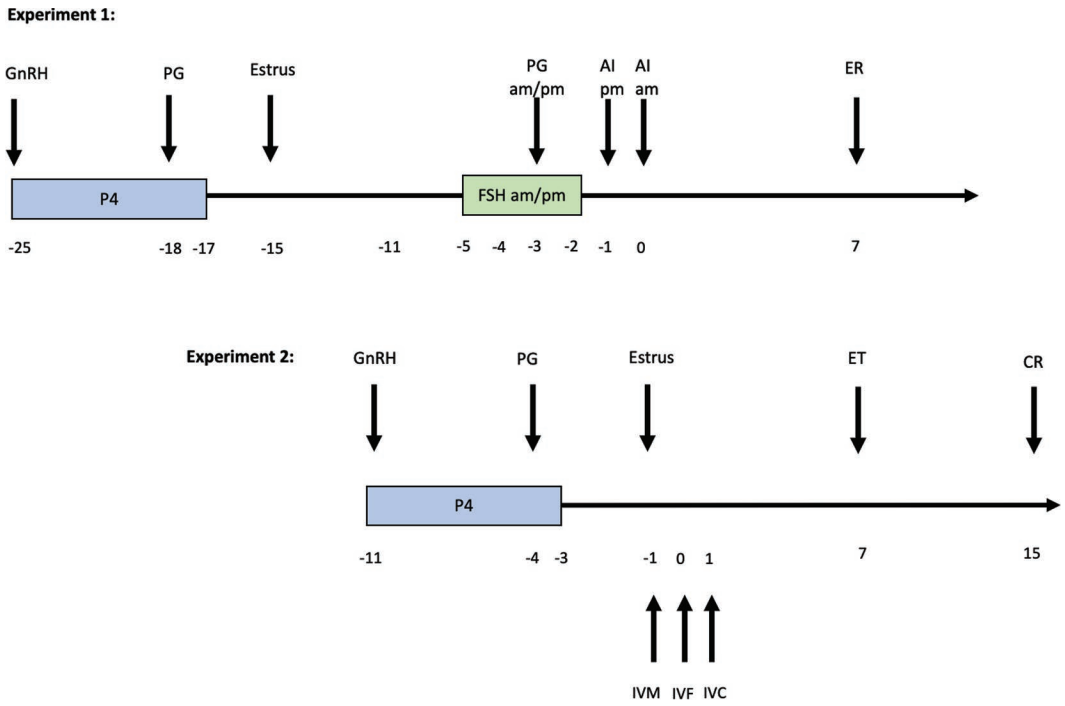

Figure 2. Protocol for superovulation of heifers in experiment 1 and for in vitro embryo generation and transfer in experiment 2. P4 = progesterone; PG = prostaglandin; ER = embryo recovery; IVM = in vitro maturation; IVF = in vitro fertilization; IVC = in vitro culture; ET = embryo transfer; CR = conceptus recovery.

covered structures (unfertilized oocytes/embryos) were classified according to developmental stage, following the guidelines of the International Embryo Technology Society, mounted on glass slides, fixed in 100% ethanol, and stained with 4′,6-diamidino-2-phenylindole (Sigma-Aldrich) at a concentration of 1 μg/mL to assess the number of cells and accessory sperm (Nikon Eclipse TE2000, 400×), the latter as a proxy for the number of sperm at the site of fertilization (Saacke, 2008).

Experiment 2. Effect of Sire Fertility Status on Conceptus Development to d 15

The aim of this experiment was to evaluate the effect of sire fertility on survival of bovine embryos to d 15. Charolais- or Limousin-cross heifers (n = 42, 24.3 mo ± 4.7, 608 kg ± 27.9) were synchronized as described above. Blastocysts were produced in vitro following IVF with semen from the same HF (n = 3) and LF (n = 3) bulls used in experiment 1. See experimental design in Figure 2.

Briefly, immature cumulus-oocyte complexes were recovered by aspirating follicles from the ovaries of heifers and cows slaughtered at a local abattoir, washed in PBS, and matured for 24 h in groups of 50 in 500 μL of TCM-199 supplemented with 10% fetal calf serum and 10 ng/mL epidermal growth factor at 39°C under an atmosphere of 5% CO_2 in air with maximum humidity. Matured cumulus-oocyte complexes were inseminated with frozen-thawed Percoll-separated motile bull sperm at a concentration of 1×10^6 sperm/mL from each of the HF or LF bulls. Gametes were co-incubated for 20 h at 39°C in an atmosphere of 5% CO_2 in air with maximum humidity. Presumptive zygotes were denuded by gentle vortexing and cultured in synthetic oviduct fluid droplets (25-μL droplets under mineral oil; 25 embryos per droplet) at 39°C in a humidified atmosphere with 5% CO_2 and 5% O_2. On d 7 (IVF = d 0), good quality blastocysts were removed from culture, pooled per bull, washed in PBS, loaded into straws (5–10 embryos per straw), and transferred to synchronized recipients on d 7 of the estrous cycle (7 recipients per bull). The mean (±SE) number of embryos transferred per recipient was 8.33 ± 0.41 for HF bulls, 8.57 ± 0.38 for LF bulls, and 8.45 ± 0.28 overall. All heifers were slaughtered on d 15. Reproductive tracts were recovered, transported to the laboratory within 2 h of slaughter, and gently dissected. Each uterine horn was gently flushed with 20 mL of PBS containing 5% fetal calf serum. The number and dimensions (length and width) of recovered conceptuses were recorded. Conceptus recovery rate was considered to be synonymous with conceptus survival.

When flushing the uterus on d 15 (following slaughter, as in this study), the likelihood of not finding a conceptus if one is present is very low in our experience given the size and nature of the conceptus at this stage.

Statistical Analysis

Data were checked for normality and homogeneity of variance using histograms, qqplots, and formal statistical tests in the Univariate procedure (version 9.1.3, SAS Institute Inc.). Data that were not normally distributed (conceptus length data) were transformed by raising the variable to the power of lambda. The appropriate lambda value was obtained by conducting a Box-Cox transformation analysis using the TRANSREG procedure of SAS.

In experiment 1, recovered structures (oocytes/embryos) were classified according to developmental stage. A binary distribution was used by coding a particular stage as 1, and all other structures were coded as 0 and analyzed using the LOGISTIC function of the GENMOD procedure in SAS. In addition, all blastocyst stage embryos were also binned into an "advanced stages of development" category for analysis purposes. Analysis of the accessory sperm number and cell number data was undertaken using PROC GENMOD assuming a logit link function and a Poisson distribution. The model contained the fixed effects of bull fertility (high or low), follicle number at the time of AI was included as a co-variate, and donor heifer and donor heifer × bull fertility interaction were included as random terms.

In experiment 2, embryo dimension and morphology data were analyzed using procedure Mixed of SAS with a model that included fixed effect of bull fertility (high or low), and recipient heifer and recipient heifer × bull fertility interaction were included as random terms. Differences among means were determined by *F*-tests using Type III sums of squares. The PDIFF option and the Tukey test were applied to evaluate pairwise comparisons between means. Conceptus recovery rate was analyzed using the GENMOD procedure in SAS. For each individual heifer, conceptus recovery rate was calculated as the number of recovered structured over the number of transferred embryos. The model contained the fixed effects of bull fertility (high or low), and recipient heifer and recipient heifer × bull fertility interaction were included as random terms. A binomial distribution was assumed with a logit link function. Mean values were considered to be different when $P < 0.05$ and considered a tendency when $P \geq 0.05$ and <0.10.

RESULTS

Experiment 1. Effect of Sire Fertility Status on Fertilization Rate and Early Embryo Development

Data are presented in Table 1. As per the design of the study, the mean (±SEM) number of CL per superovulated donor was not different between groups (HF: 17.4 ± 8.2 vs. LF: 17.0 ± 8.4; $P > 0.05$). Overall recovery rate (total structures/total CL) was 52.6% and was not different between HF (49.7%) and LF (55.3%) bulls ($P > 0.05$). Mean (±SEM) number of embryos recovered per recipient was 8.7 ± 5.2 and 9.4 ± 5.5 for HF and LF, respectively ($P > 0.05$). Overall fertilization rate of recovered structures was 95.9% and was not different between groups (HF: 94.9% vs. LF: 96.8%). The percentages of unfertilized oocytes (5.1 vs. 3.2%, $P > 0.05$), morulae (14.1 vs. 35.1%, $P < 0.01$), early blastocysts (6.4 vs. 0%, $P > 0.05$), blastocysts (32.1 vs. 22.3%, $P > 0.05$), and expanded blastocysts (42.3 vs. 39.4%, $P > 0.05$) between HF and LF bulls are presented in Table 1. A greater proportion of embryos were at a more advanced stage of development (early blastocyst, blastocyst, and expanded blastocyst combined) at d 7 following AI with semen from HF versus LF bulls ($P < 0.05$).

One blastocyst was lost during processing and is not included in the embryo cell number or accessory sperm number data. Mean (±SEM) embryo cell number on d 7 was greater for HF (91.5 ± 2.70) versus LF (78.0 ± 3.00) bulls ($P < 0.05$). Mean (±SEM) embryo cell number increased with advancing developmental stage: morula (62.0 ± 3.40), early blastocyst (80.0 ± 5.24), blastocyst (79.6 ± 3.30), and expanded blastocyst (101.1 ± 2.57; Table 1). There was a tendency for more cells in morulae ($P = 0.08$) and more cells in blastocysts from HF versus LF bulls. Overall, 17% of recovered structures had at least one accessory sperm (HF: 23.1% vs. LF: 11.8%, $P < 0.05$). Accessory sperm were detected on all the different classes of structures recovered (unfertilized oocytes: 4/7; morula: 5/44; early blastocyst: 2/5; blastocyst: 16/46; expanded blastocyst: 2/70). Number of accessory sperm was highly variable (range HF: 0 to 45; LF: 0 to 8; $P < 0.01$). Mean (±SEM) number of accessory sperm was greater for embryos derived from HF (2.9 ± 1.03) than for LF 0.34 ± 0.13 bulls ($P < 0.05$). Corresponding median values were both zero. Taking into account only those structures with accessory sperm, the mean number of sperm per structure was 12.7 ± 3.66 versus 2.9 ± 0.75 ($P < 0.05$) for HF and LF bulls, respectively, with corresponding median values of 3.5 and 2, respectively.

Table 1. Summary data from experiment 1 (values represent mean ± SE unless otherwise stated)

Item	High fertility	Low fertility	Total	High vs. low fertility
Number of corpora lutea per superovulated donor (range)	17.4 ± 8.2 (7–34)	17.0 ± 8.4 (5–30)	17.2 ± 8.1 (5–34)	0.23
Recovery, % (n/total)	40.7 (78/157)	55.3 (94/170)	52.6 (172/327)	0.52
Fertilization, % (n/total)	94.9 (74/78)	96.8 (91/94)	95.9 (165/172)	0.11
Unfertilized oocyte, % (n/total)	5.1 (4/78)	3.2 (3/94)	4.1 (7/172)	0.52
Morula, % (n/total)	14.1 (11/78)	35.1 (33/94)	25.9 (44/172)	**
Early blastocyst, % (n/total)	6.4 (5/78)	0 (0/94)	2.9 (5/172)	—
Blastocyst, % (n/total)	32.1 (25/78)	22.3 (21/94)	26.7 (46/172)	0.15
Expanded blastocyst, % (n/total)	42.3 (33/78)	39.4 (37/94)	40.7 (70/172)	0.69
Morula cell number (n)	72.5 ± 7.79 (11)	58.6 ± 3.58 (33)	62.0 ± 3.4 (44)	†
Early blastocyst cell number (n)	80 ± 5.24 (5)	0	80.0 ± 5.24 (5)	—
Blastocyst cell number (n)	86.9 ± 4.59 (25)	70.6 ± 3.96 (20)	79.6 ± 3.30 (45)	**
Expanded blastocyst cell number (n)	103 ± 3.03 (33)	99.3 ± 4.07 (37)	101.1 ± 2.57 (70)	0.24
Embryo cell number on d 7 (n)	91.5 ± 2.70 (74)	78.0 ± 3.00 (90)	84.1 ± 2.11 (164)	***
Structures with accessory sperm, % (n/total)	23.1 (18/78)	11.8 (11/93)	17.0 (29/171)	*
Number of accessory sperm[1] (n)	2.9 ± 1.03 (78)	0.34 ± 0.13 (93)	1.5 ± 0.48 (171)	*
Number of accessory sperm[2] (n)	12.7 ± 3.66 (18)	2.9 ± 0.75 (11)	8.97 ± 2.44 (29)	*

[1]Based on all structures recovered.

[2]Based only on those structures with accessory sperm.

†$P = 0.08$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Experiment 2. Effect of Sire Fertility Status on Conceptus Development to d 15

Overall conceptus recovery rate on d 15 was 52.1% (185/355). The mean (±SE) number of conceptuses recovered per recipient on d 15 was 4.95 ± 0.57 for HF bulls, 3.85 ± 0.51 for LF bulls, and 4.40 ± 0.39 overall. Conceptus recovery rate was higher in HF (59.4%, 104/175) versus LF (45.0%, 81/180; $P < 0.05$). Variation in length of all recovered conceptuses is summarized in Figure 3. Overall, mean (±SEM) conceptus length was 24.4 ± 2.0 mm. Mean (±SEM) length of recovered conceptuses for HF bulls was 24.3 ± 2.3 and 24.5 ± 3.5 mm for LF bulls with a range of 0.5 to 123 mm and 0.5 to 220 mm in length, respectively, and was not affected by fertility status ($P < 0.05$; Figure 3). Within the HF group there was an effect of individual bull on conceptus length ($P < 0.001$), whereas within LF bulls there was no such effect (Figure 3).

DISCUSSION

Even among the highly selected bull population in AI centers, where semen quality is scrutinized before release, significant variation exists in field fertility. However, the utility of standard laboratory tests to reliably identify bulls of lower than average fertility is limited. Using 2 different models to assess aspects of sperm function, the aim of this study was to compare fertilization rate, early embryo development, and post-hatching conceptus elongation following insemination with sperm from bulls exhibiting extremes of fertility within an AI bull population. The end points of d 7 (experiment 1) and d 15 (experiment 2) were chosen because they represent key checkpoints in development. Notwithstanding a high fertilization rate, a significant proportion of embryos are no longer viable by d 7 in high-producing dairy cows (Sartori et al., 2010; Wiltbank et al., 2016). In addition, failure of the conceptus

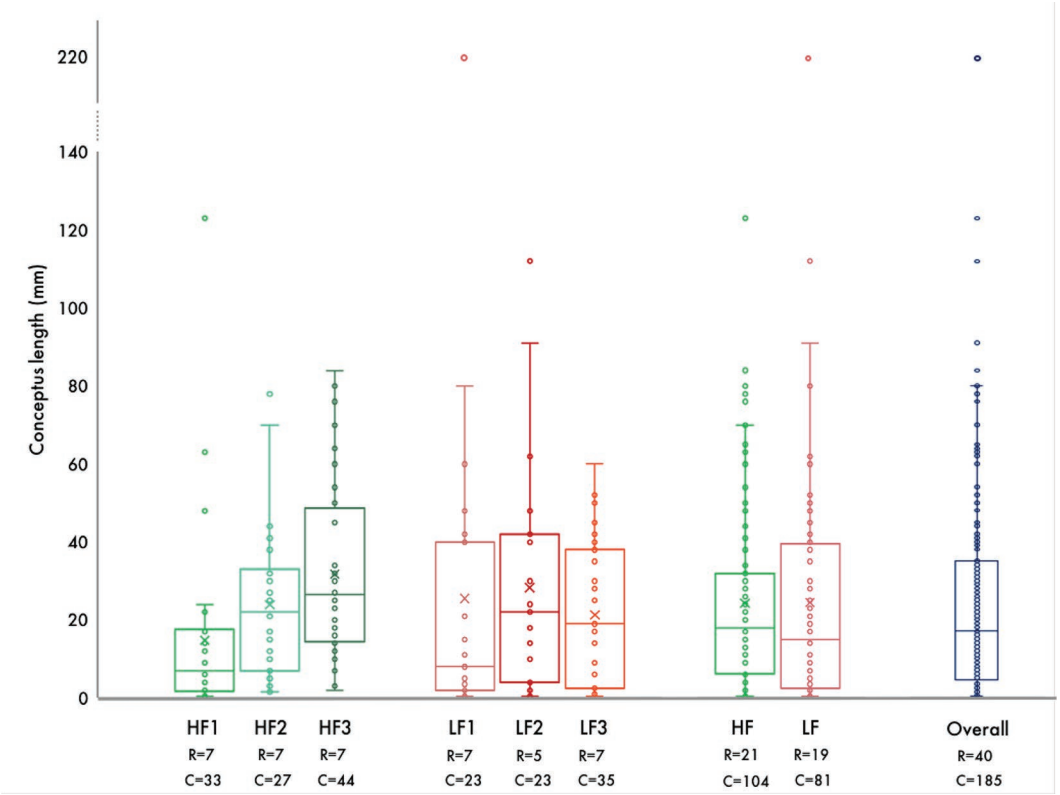

Figure 3. Box plots illustrating the variation in d-15 conceptus (C) length from all recipients (R), produced from high fertility bulls (HF1, HF2, HF3) and low fertility bulls (LF1, LF2, LF3). Each bull was represented by n = 7 heifers except for LF2, which had n = 5. One heifer was removed from the study due to a tract infection, and no conceptuses were recovered from another tract. Each box represents the interquartile range (IQR, 25th to the 75th percentile) with the median value represented by a horizontal line and the mean denoted by an X. Maximum (quartile 3 + 1.5 × IQR) and minimum (quartile 1 − 1.5 × IQR) values are denoted by the whiskers. Outliers (>1.5 times the IQR) are also shown.

to undergo appropriate elongation is associated with an altered conceptus transcriptome (Barnwell et al., 2016), low interferon-tau secretion (Rizos et al., 2012), and failure to elicit an appropriate response from the endometrial transcriptome (Sánchez et al., 2019), potentially leading to failure of maternal recognition of pregnancy.

The main findings of the study are that (1) differences in field fertility among AI sires were not reflected in fertilization rate; (2) a greater proportion of embryos were at a more advanced stage of development at d 7 following AI with semen from HF versus LF bulls; (3) mean embryo cell number on d 7 was greater in embryos derived from HF versus LF bulls; (4) the number of sperm in the vicinity of the oocyte at fertilization, as assessed by accessory sperm number, was greater in HF bulls, and (5) despite no difference in the mean length of recovered conceptuses, the proportion of conceptuses surviving to d 15 was greater in HF bulls.

Despite the undeniable contribution of the sire to embryo loss, few studies have attempted to characterize when precisely this loss occurs. Using sires associated with high or low incidence of pregnancy loss (between d 30 and 100 of gestation), Franco et al. (2018) investigated their effect on circulating concentrations of pregnancy-associated glycoproteins (**PAG**) in multiparous Nelore cows; PAG concentrations reflected probability of pregnancy maintenance and were influenced by both sire and sire breed used (Franco et al., 2018). Subsequently, Franco et al. (2020) evaluated sire contribution to pregnancy loss in different periods of embryonic and fetal development of beef cows. Overall early embryo mortality (between d 24 and 31) was 5.54% (1.8 to 11.7%), whereas late embryo mortality/fetal death (between d 31 and 60) was 6.7% (2.3 to 12.6%). These studies did not evaluate early embryo mortality earlier than 24 d and used indirect methods to assess loss (progesterone concentrations, PAG). Here, to characterize the sire's contribution to fertilization failure and early embryo/conceptus survival, we used direct methods of embryo recovery to assess early embryo development as well as conceptus survival to give a broader understanding of whether events along the early developmental axis leading to pregnancy establishment are implicated.

The use of superovulated heifers in experiment 1 allowed more observations per heifer than in a single-ovulating scenario. While altered sperm transport has been reported following superovulation (Hyttel et al., 1991), it is important to note that, in the present study, any alterations would be the same for both treatments. In addition, fertilization and embryo development rates of recovered embryos were high in both groups. In contrast to these observations, Ortega et al. (2018) reported that, in superovulated heifers, high fertility bulls produced a lower percentage of unfertilized oocytes and fewer degenerated embryos compared with low fertility bulls. Consistent with those observations, in the current study, more embryos were at the morula stage and fewer were at more advanced stages (blastocyst, expanded blastocyst) following AI with semen from LF versus HF bulls. Indeed, even among embryos classified as morulae, mean number of cells per embryo was higher in embryos derived from HF bulls, indicative of embryos of superior quality.

While accessory sperm are not directly involved in fertilization, they represent a population of sperm capable of successfully traversing the female reproductive tract and partially penetrating the zona pellucida and as such can be used as a proxy for the number of sperm in the vicinity of the oocyte around fertilization (Saacke, 2008). The number of accessory sperm in the zona pellucida has been positively correlated with fertility in cattle (Hunter and Wilmut, 1984; Hawk and Tanabe, 1986), sheep (Hawk and Cooper, 1984), swine (Pursel, 1982; Weitze et al., 1988), and rabbits (Overstreet and Adams, 1971). In artificially inseminated cattle, DeJarnette et al. (1992) have shown that number of accessory sperm is also positively related to embryo quality 6 d after insemination. Number of accessory sperm on oocytes recovered from cows after mating was not correlated with in vitro competitive binding of the spermatozoa (Braundmeier et al., 2002). However, in a series of studies summarized by Saacke (2008), over 1,000 ova/embryos from single-ovulating cows 6 d after AI were assessed (involving approximately 30 bulls). The distribution of accessory sperm in the zona pellucida was very skewed, with an average, median, and mode of 12, 2.4, and 0 sperm per oocyte/embryo, respectively, indicating that only a few sperm compete for fertilization at a given time. Furthermore, embryo quality was associated with median accessory sperm number; good to excellent embryos had a higher accessory sperm number than degenerate or fair to poor embryos, but the mode remained 0, regardless of embryo quality. These data are consistent with the observations in the present study where, overall, only 17% of recovered structures had at least one accessory sperm. Number of accessory sperm was highly variable (range HF: 0 to 45; LF: 0 to 8); nonetheless, for those structures with accessory sperm, the mean number of sperm per structure was greater in HF (12.7 ± 3.66) compared with LF (2.9 ± 0.75) bulls, suggesting that more sperm reached the site of fertilization.

Consistent with superior embryo quality observed on d 7 described above, in experiment 2, conceptus survival to d 15 was greater in HF (59.4%) compared with LF (45.0%) bulls. Conceptus elongation is a critical process in the development of the ruminant embryo and is a prerequisite for maternal pregnancy recognition and initiation of implantation. Large variation exists in the length of age-matched conceptuses and this has been associated with likely viability of such conceptuses. In particular, short conceptuses exhibit a different transcriptome to long conceptuses (Barnwell et al., 2016) and, importantly, elicit a different response from the endometrium (Sánchez et al., 2019), mainly associated with lower concentrations of interferon-tau production (Rizos et al., 2012). Thus, conceptus length on a given day during the period of elongation around maternal recognition of pregnancy can be used as an indicator of conceptus health and developmental competence and is reflective of likelihood of conceptus survival and pregnancy maintenance.

To our knowledge, the only other study that directly investigated the effect of sire fertility on conceptus elongation is that by Ortega et al. (2018), who investigated sire influence on pregnancy establishment in cattle. In agreement with our observations, in that study, conceptus length on d 16 was not different between groups following transfer of 3 to 5 in vivo–produced embryos from either high or low fertility sires on d 7 postestrus. Furthermore, the conceptus transcriptome was not appreciably different between high and low fertility sires. Thus, data would suggest that whereas fewer conceptuses survived to d 15 (present study), those that did survive were comparable. Bull fertility status has been associated with alterations in the sperm transcriptome (Feugang et al., 2010), DNA methylation signatures in sperm (Kropp et al., 2017), as well as the metabolome and proteome of sperm and seminal plasma (Memili et al., 2020), all of which could potentially have consequences for embryo development beyond fertilization. At fertilization, the sperm delivers more than just paternal DNA, but rather a package including RNAs, transcription factors, and cell signaling molecules (Krawetz, 2005). Several studies have assessed the mRNA expression of proteins associated with sperm function in bulls of differing fertility (Arangasamy et al., 2011; Kasimanickam et al., 2012). Furthermore, Kropp et al. (2017) related differences in sperm methylation profiles between sires of varying fertility with altered transcriptomic profiles in preimplantation embryos.

Thus, the consequences of altered sperm function may not be seen until long after fertilization.

CONCLUSIONS

In conclusion, although differences in field fertility among AI sires used in this study were not reflected in fertilization rate, differences in embryo quality were apparent by d 7. These differences likely contributed to the higher proportion of conceptuses surviving to d 15 in HF bulls. While there was large variation in number of accessory sperm within and across groups, nonetheless, fewer accessory sperm were recorded following insemination with LF bulls. Further molecular characterization of paternal factors contributing to early embryo development may lead to the identification of biomarkers for better selection of sires to improve reproductive efficiency.

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