



Original Research Article

Optimizing Holstein bovine oocyte development: Evidence-Based donor selection for OPU-IVP

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ABSTRACT

Ovum pick-up and in vitro embryo production (OPU-IVP) in Holstein cows is influenced by donor characteristics, yet quantitative selection criteria for optimizing oocyte developmental competence remain limited. In this study, data from 600 multiparous Holstein cows across three commercial dairy farms were analyzed to evaluate the effects of body condition score (BCS), age, parity, days in milk (DIM), and milk yield on oocyte quality and subsequent embryonic development. Oocyte developmental competence was highest in donors with BCS of 2.75–3.25, age ≤ 80 months, parity ≤ 4 , and DIM 50–70 days, whereas underconditioned (BCS ≤ 2.5) or overconditioned cows (BCS ≥ 3.75), older cows, or those beyond the fourth parity exhibited lower proportions of grade A/B cumulus-oocyte complexes and blastocyst formation. Postpartum interval further affected outcomes, with oocytes collected at 50–70 DIM showing superior maturation, fertilization, cleavage, and blastocyst rates compared with those collected at 30–40 DIM. In contrast, daily, weekly average, or 305-day milk yield did not significantly influence any measure of oocyte developmental competence. These findings indicate that BCS, age, parity, and DIM are key determinants for donor selection in OPU-IVP, and applying these quantitative criteria provides a practical framework for improving embryo production efficiency in commercial Holstein herds.

1. Introduction

Ovum pick-up coupled with in vitro embryo production (OPU-IVP) is a cornerstone technology for accelerating genetic gain in dairy and beef cattle [1,2], allowing repeated collection of oocytes from genetically superior females and thereby shortening generation intervals while enhancing the dissemination of desirable traits [3]. Despite these advantages, the efficiency of OPU-IVP remains highly variable in commercial herds, primarily due to inconsistent oocyte quality and developmental competence among donor cows. Previous studies have largely focused on single donor characteristics under controlled experimental conditions, providing limited guidance for field applications. A systematic, field-based evaluation of multiple donor factors is therefore needed to establish quantitative selection criteria that can improve the predictability and efficiency of OPU-IVP programs [4].

The developmental potential of oocytes retrieved by OPU is influenced by a complex interplay of intrinsic and extrinsic factors. Among these, the physiological and productive status of the donor—including body condition score (BCS), age, parity, days in milk (DIM), and milk yield—is known to influence ovarian function and oocyte quality [5–7]. BCS, as an indicator of energy balance, is closely linked to reproductive performance, with both excessive thinness and obesity associated with impaired follicular development and compromised oocyte competence [8,9]. Age and parity serve as proxies for reproductive aging, as older cows typically exhibit reduced ovarian reserve and increased oocyte aneuploidy [10]. DIM reflects the dynamic process of postpartum uterine involution and metabolic adaptation, which may affect the follicular microenvironment [11]. Milk yield, particularly in high-producing dairy cows, has been hypothesized to negatively impact fertility due to the metabolic demands of lactation [12].

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Despite the recognized importance of these factors, most previous studies have examined them in isolation or under controlled experimental conditions that may not accurately represent commercial herd environments, limiting their applicability for practical donor selection. For example, large scale phenotypic analyses in Holstein populations have demonstrated significant variability in OPU-IVP outcomes related to donor age and seasonal effects, but practical, quantitative selection criteria are still lacking [13]. Similarly, breed and management differences have been shown to affect oocyte and embryo yields, highlighting the complexity of donor effects in real-world settings [14]. Consequently, comprehensive, field-based evaluations that simultaneously assess multiple donor characteristics are needed to derive reliable selection criteria. Such criteria would enable veterinarians and embryologists to identify optimal donors with greater confidence, thereby improving the predictability, efficiency, and cost-effectiveness of commercial OPU-IVP programs.

The present study aimed to systematically evaluate the relationships between donor BCS, age, parity, DIM, and milk yield and their effects on oocyte developmental competence in Holstein cows undergoing OPU-IVP. Furthermore, the study sought to establish quantitative, evidence-based donor selection thresholds that could be applied in commercial embryo production programs, thereby improving both the efficiency and predictability of embryo yields and providing practical guidance for veterinarians and embryologists in identifying optimal donors.

2. Materials and methods

All experimental procedures were approved by the Animal Care and Use Committee of Northwest A&F University and conducted in accordance with national animal welfare regulations.

2.1. Experimental farms and animals

This study was conducted from March 2022 to December 2024 at three large-scale commercial dairy farms located in Ningxia, Gansu, and Shaanxi provinces, China. Farm characteristics and management practices are summarized in [Supplementary Table S1](#). Briefly, all farms employed total mixed ration feeding three times daily, milking three times daily at 8-h intervals, and digital herd management using the "Yimuyun" system. Throughout the study period, herd structure, diet formulation, and management routines remained stable, and no outbreaks of infectious or metabolic diseases occurred.

A total of 600 multiparous Holstein cows in the voluntary waiting period (30–70 DIM) were enrolled. Each donor underwent 2 OPU sessions during this period. To isolate the effect of each donor factor, a controlled variable design was employed: when evaluating a given factor (e.g., BCS), groups were matched for all other background characteristics (age, parity, DIM, milk yield) to minimize confounding. For each analysis, donors were stratified by the factor of interest, and groups were balanced for the remaining four factors using propensity score matching or frequency matching to ensure comparable baseline characteristics across groups.

2.2. Body condition scoring

BCS was assessed by trained personnel using a 5-point scale with 0.25-point increments, where 1 = emaciated and 5 = obese [15]. Evaluations were based on visual appraisal and palpation of eight anatomical regions: pelvic area, hook, pin bones, ribs, sacral ligament, tailhead ligament, hip joint, and short ribs. To ensure consistency, a folding protractor was used for objective calibration when necessary. For analysis, cows were stratified into nine BCS categories ranging from 2 to 4.

2.3. Ovum pick-up

OPU was performed as previously described with minor modifications [16]. Briefly, donors received an ovarian superstimulation protocol tailored to the presence or absence of a corpus luteum, involving dominant follicle aspiration, prostaglandin $F_{2\alpha}$ (25 mg, i.m.; Ningbo Sansheng, China), and FSH (100 IU, i.m., every 12 h for five injections; Ningbo Sansheng). OPU was conducted 36 h after the final FSH injection.

Cows were restrained in a chute, and caudal epidural anesthesia was administered using 5 mL of 2% lidocaine hydrochloride. The perineal region was cleaned and disinfected. A portable ultrasound system (BCF Easi-Scan, IMV Technologies, France) equipped with a 7.5 MHz convex transducer and a 18 G single-lumen needle (1.2 × 80 mm) was used. The needle was inserted vertically (90°) to a depth of one-third to one-half of the follicular diameter, with the bevel oriented downward. Follicles ≥ 2 mm were aspirated under a vacuum pressure of 50 mmHg (flow rate: 10–15 mL/min). Follicular aspirates were collected into 50-mL conical tubes containing pre-warmed (37 °C) PBS supplemented with 3% (v/v) fetal bovine serum (FBS; Gibco, USA).

2.4. In vitro embryo production

2.4.1. COCs grading and in vitro maturation (IVM)

Cumulus-oocyte complexes (COCs) were isolated from follicular aspirates using a 75-μm filter and graded morphologically under a stereomicroscope according to the Chinese national standard (GB/T 45202): Grade A, ≥ 5 compact cumulus cell layers and homogeneous cytoplasm; Grade B, 3–5 cumulus layers and homogeneous cytoplasm; Grade C, 1–3 cumulus layers or heterogeneous cytoplasm; Grade D, expanded cumulus or denuded oocytes. Only Grade A and B COCs were selected for IVM.

IVM was performed in 100-μL microdrops of oocyte maturation medium (OM) consisting of TCM-199 (Gibco) supplemented with 10% (v/v) FBS, 1 μg/mL estradiol, 0.075 IU/mL hMG, 20 ng/mL bFGF, 20 ng/mL EGF, and 1% (v/v) ITS. Microdrops were overlaid with mineral oil and equilibrated at 38.5 °C in 5% CO₂ in air for at least 2 h. COCs were washed in PBS and OM, then transferred to microdrops (10–15 COCs per drop) and cultured for 22–24 h.

2.4.2. In vitro fertilization (IVF)

Frozen-thawed semen from a single proven Holstein sire was used throughout the study. Motile spermatozoa were isolated by Percoll density gradient centrifugation (45%/90%). Motile spermatozoa were isolated by Percoll density gradient centrifugation. Briefly, 2 mL of 90% Percoll solution was placed at the bottom of a 15-mL centrifuge tube, and 2 mL of 45% Percoll solution was carefully layered on top to form a discontinuous gradient. The gradient was equilibrated at 38.5 °C in 5% CO₂ for at least 2 h. Frozen-thawed semen was layered onto the gradient and centrifuged at 1470 rpm (approximately 350 × g) for 10 min at room temperature. The supernatant was discarded, and the sperm pellet was resuspended in 1 mL of TL-Hepes medium and centrifuged at 1380 rpm (approximately 300 × g) for 5 min. The final sperm pellet was resuspended in approximately 100 μL of TL-Hepes medium for subsequent use. Matured COCs were partially denuded by gentle pipetting and washed in TL-Hepes medium. Groups of 20–25 COCs were transferred to 100-μL microdrops of IVF medium (BO medium supplemented with 6 mg/mL BSA and 10 μg/mL heparin). Spermatozoa were added at a final concentration of 1.5–2.0 × 10⁶/mL, and gametes were co-incubated for 10–12 h at 38.5 °C in 5% CO₂ in air.

2.4.3. In vitro culture

Following IVF, presumptive zygotes were denuded of remaining cumulus cells and spermatozoa by vortexing in hyaluronidase solution, washed in synthetic oviductal fluid (SOF) medium, and cultured in 100-μL microdrops of SOF supplemented with 8 mg/mL BSA, 2% (v/v)

essential amino acids, and 1% (v/v) non-essential amino acids. Culture was performed at 38.5 °C in a humidified atmosphere of 5% CO₂, 5% O₂, and 90% N₂. Cleavage rate was assessed at 48 h post-insemination, and blastocyst rate was evaluated on Day 7.

2.5. Outcome measures

The primary outcome measures were: (1) proportion of grade A/B COCs (number of grade A/B COCs/total COCs recovered); (2) maturation rate (number of oocytes extruding the first polar body/number of COCs cultured minus degenerated oocytes); (3) fertilization rate (number of oocytes with two pronuclei/number of matured oocytes); (4) cleavage rate (number of cleaved embryos/number of matured oocytes); and (5) blastocyst rate (number of blastocysts/number of cleaved embryos).

2.6. Statistical analysis

Data were analyzed using SPSS Statistics 25 (IBM Corp., USA) and GraphPad Prism 10.1.3 (GraphPad Software, USA). All values are expressed as means $\pm$ standard error of the mean (SEM). Comparisons among groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test, consistent with standard practice in published OPU-IVP studies [12,17]. Differences were considered statistically significant at $p < 0.05$. Different superscript letters within columns denote significant differences between groups. To verify the robustness of our findings, we performed two complementary analyses for all primary outcomes. First, linear regression models were fitted with donor-level percentages as the dependent variable and donor categories as independent variables. Residual normality was assessed using the Shapiro-Wilk test and quantile-quantile (Q-Q) plots, and homoscedasticity was evaluated with the Breusch-Pagan test. Where residual diagnostics indicated deviation from normality, arcsine square-root transformation was applied to the percentage data. Second, the Kruskal-Wallis non-parametric test was performed as an additional safeguard against potential violations of the normality assumption. The results of both the linear regression and Kruskal-Wallis analyses were entirely consistent with the ANOVA findings, confirming the same pattern of significant differences among groups.

3. Results

3.1. BCS and oocyte developmental competence

BCS exhibited a pronounced curvilinear relationship with all measures of oocyte developmental competence in Holstein cows undergoing OPU-IVP. Both the proportion of grade A/B COCs and subsequent embryonic development—reflected in maturation, fertilization, cleavage, and blastocyst rates—improved progressively as BCS increased from 2 to

the optimal range of 2.75–3.25, after which all parameters declined sharply at higher scores (≥ 3.5). Donors with moderate BCS consistently produced higher-quality oocytes and greater blastocyst yields compared with underconditioned (BCS ≤ 2.5) or overconditioned cows (BCS ≥ 3.75), highlighting the detrimental effects of both negative and positive energy balance on follicular development. Importantly, this suggests that selecting donors within the optimal BCS range can enhance the efficiency and predictability of OPU-IVP programs, providing a practical criterion for commercial embryo production ($p < 0.05$, Table 1).

3.2. Donor age and oocyte developmental competence

Donor age exerted a significant influence on oocyte quality and subsequent embryonic development in Holstein cows undergoing OPU-IVP. Cows aged 20–80 months maintained high proportions of grade A/B COCs and stable blastocyst formation, indicating robust developmental competence across this age range. In contrast, donors older than 80 months showed a pronounced decline in all developmental parameters, including COC quality, maturation, fertilization, cleavage, and blastocyst rates ($p < 0.05$, Table 2). Furthermore, advanced donor age may exacerbate the negative effects of suboptimal BCS or high parity, collectively compromising embryo production efficiency. These findings underscore the importance of prioritizing younger donors in OPU-IVP programs to maximize both oocyte quality and embryo yield, providing a practical criterion for selection in commercial dairy operations.

3.3. Parity and oocyte developmental competence

Parity exerted a notable influence on oocyte quality and fertilization outcomes in Holstein cows undergoing OPU-IVP. Cows in their first to fourth parity maintained high and stable developmental competence, reflected in robust proportions of grade A/B cumulus-oocyte complexes, maturation, and fertilization rates, as well as consistent blastocyst formation. A decline in oocyte quality was first observed at the fifth parity, with reductions in COC grade and fertilization efficiency, and further deterioration occurred at the sixth parity, accompanied by a numerical decrease in blastocyst yield ($p < 0.05$, Table 3). This pattern is consistent with cumulative reproductive aging and physiological stress associated with repeated lactations, which may compromise follicular development and oocyte competence. These findings highlight the importance of considering parity in donor selection for OPU-IVP programs, suggesting that cows in earlier parities are likely to maximize embryo production efficiency in commercial herds.

3.4. DIM and oocyte developmental competence

DIM had a progressive and significant impact on all measured outcomes (Table 4). Oocyte quality and developmental competence were

Table 1
Correlation analysis between donor BCS and the developmental potential of oocytes in Holstein multiparous cows.

BCS	Donors (n)	OPU sessions (n)	COC/donor (n)	Grade A/B COCs (%)	Maturation (%)	Fertilization (%)	Cleavage (%)	Blastocyst (%)
2	58	116	15.88	51.44 $\pm$ 1.18 ^a	67.7 $\pm$ 0.89 ^a	63.2 $\pm$ 0.94 ^a	63.2 $\pm$ 1.26 ^a	25.68 $\pm$ 0.81 ^a
2.25	66	132	16.80	53.44 $\pm$ 0.84 ^a	71.05 $\pm$ 1.34 ^a	65.15 $\pm$ 0.83 ^{ad}	74.19 $\pm$ 0.98 ^{ab}	30.96 $\pm$ 0.89 ^{ab}
2.5	78	156	16.68	55.40 $\pm$ 1.19 ^{ab}	75.03 $\pm$ 0.77 ^{ad}	72.23 $\pm$ 0.61 ^{de}	75.83 $\pm$ 0.94 ^{bf}	38.57 $\pm$ 1.30 ^{bd}
2.75	83	166	16.35	59.96 $\pm$ 0.86 ^{bd}	83.56 $\pm$ 1.01 ^{bd}	83.1 $\pm$ 0.75 ^{be}	83.69 $\pm$ 1.09 ^{ef}	41.95 $\pm$ 1.16 ^c
3	75	150	17.14	59.83 $\pm$ 1.22 ^{bd}	83.78 $\pm$ 1.25 ^b	81.88 $\pm$ 1.03 ^b	84.13 $\pm$ 0.81 ^c	39.59 $\pm$ 1.14 ^c
3.25	88	176	15.38	59.72 $\pm$ 1.48 ^{bd}	84.38 $\pm$ 0.95 ^b	82.53 $\pm$ 0.62 ^b	82.79 $\pm$ 0.81 ^c	37.32 $\pm$ 0.62 ^{bcd}
3.5	67	134	17.38	56.07 $\pm$ 1.37 ^{ad}	74.51 $\pm$ 0.88 ^{ad}	74.43 $\pm$ 0.78 ^d	75.05 $\pm$ 1.05 ^{bd}	35.98 $\pm$ 0.93 ^d
3.75	54	108	16.18	51.52 $\pm$ 1.01 ^a	74.99 $\pm$ 1.41 ^{ad}	70.64 $\pm$ 0.90 ^{ad}	72.4 $\pm$ 0.64 ^{ab}	30.59 $\pm$ 0.69 ^a
4	31	62	15.79	44.59 $\pm$ 0.94 ^c	62.97 $\pm$ 0.77 ^c	63.38 $\pm$ 1.02 ^c	62.81 $\pm$ 0.77 ^c	25.17 $\pm$ 0.94 ^c

Data were presented as means $\pm$ SEM. Different letters within the same column indicate significant differences ($p < 0.05$); identical letters indicate no significant difference ($p > 0.05$). Significance was determined by one-way ANOVA with Tukey's HSD post hoc test and confirmed by linear regression analysis and the Kruskal-Wallis non-parametric test.

Table 2

Correlation analysis between donor age and the developmental potential of oocytes in Holstein multiparous cows.

Age (months)	Donors (n)	OPU sessions (n)	COC/donor (n)	Grade A/B COCs (%)	Maturation (%)	Fertilization (%)	Cleavage (%)	Blastocyst (%)
20–40	180	360	17.44	59.45 ± 1.18 ^a	82.66 ± 1.57 ^a	83.30 ± 1.76 ^a	85.67 ± 0.86 ^a	39.8 ± 1.40 ^a
40–60	173	346	18.71	62.23 ± 0.71 ^a	82.30 ± 1.05 ^a	81.63 ± 1.80 ^a	83.22 ± 0.40 ^a	40.35 ± 0.97 ^a
60–80	168	336	17.35	59.03 ± 1.68 ^a	81.34 ± 0.95 ^a	81.96 ± 1.37 ^a	86.31 ± 2.25 ^a	39.8 ± 1.61 ^a
80–100	79	158	17.20	44.61 ± 1.26 ^b	71.75 ± 1.09 ^b	71.18 ± 1.11 ^b	75.53 ± 1.31 ^b	30.1 ± 1.08 ^b

Data were presented as means ± SEM. Different letters within the same column indicate significant differences ($p < 0.05$); identical letters indicate no significant difference ($p > 0.05$). Significance was determined by one-way ANOVA with Tukey's HSD post hoc test and confirmed by linear regression analysis and the Kruskal-Wallis non-parametric test.

Table 3

Correlation analysis between donor parity and the developmental potential of oocytes in Holstein multiparous cows.

Parity	Donors (n)	OPU sessions (n)	COC/donor (n)	Grade A/B COCs (%)	Maturation (%)	Fertilization (%)	Cleavage (%)	Blastocyst (%)
1	112	224	16.93	60.29 ± 0.90 ^a	81.64 ± 1.58 ^a	87.21 ± 0.74 ^a	83.59 ± 3.21 ^a	38.74 ± 1.40 ^a
2	110	220	16.53	65.16 ± 1.22 ^a	85.30 ± 0.92 ^a	85.52 ± 0.67 ^a	90.43 ± 1.59 ^a	37.39 ± 0.89 ^a
3	131	262	18.24	63.42 ± 1.43 ^a	85.60 ± 0.89 ^a	86.46 ± 1.13 ^a	80.74 ± 3.74 ^a	40.06 ± 1.65 ^a
4	94	188	17.32	61.68 ± 1.99 ^a	82.64 ± 2.21 ^a	87.02 ± 1.71 ^a	86.39 ± 2.41 ^a	39.69 ± 0.61 ^a
5	85	170	17.15	54.60 ± 1.15 ^b	71.09 ± 2.19 ^b	73.54 ± 2.51 ^b	80.76 ± 1.94 ^a	39.22 ± 1.61 ^a
6	68	136	18.19	45.78 ± 0.64 ^c	69.67 ± 1.25 ^{bc}	69.57 ± 2.65 ^{bc}	79.94 ± 1.01 ^a	34.53 ± 1.18 ^a

Data were presented as means ± SEM. Different letters within the same column indicate significant differences ($p < 0.05$); identical letters indicate no significant difference ($p > 0.05$). Significance was determined by one-way ANOVA with Tukey's HSD post hoc test and confirmed by linear regression analysis and the Kruskal-Wallis non-parametric test.

Table 4

Correlation analysis between postpartum days of donors and the developmental potential of oocytes in Holstein multiparous cows.

DIM (d)	Donors (n)	OPU sessions (n)	COC/donor (n)	Grade A/B COCs (%)	Maturation (%)	Fertilization (%)	Cleavage (%)	Blastocyst (%)
30–40	145	290	15.43	40.99 ± 1.10 ^a	69.72 ± 2.48 ^a	68.56 ± 1.36 ^a	74.49 ± 2.91 ^a	32.59 ± 0.95 ^a
40–50	163	326	16.61	44.67 ± 0.62 ^b	73.22 ± 1.17 ^a	71.50 ± 1.75 ^a	82.42 ± 1.47 ^b	33.42 ± 1.26 ^a
50–60	174	348	17.01	54.45 ± 0.41 ^c	81.04 ± 1.61 ^b	79.67 ± 2.21 ^b	82.61 ± 1.45 ^{bc}	35.89 ± 0.95 ^{ab}
60–70	118	236	16.30	56.70 ± 1.09 ^{cd}	83.61 ± 1.59 ^{bc}	82.69 ± 1.23 ^{bc}	87.00 ± 0.84 ^{bc}	39.71 ± 0.50 ^b

Data were presented as means ± SEM. Different letters within the same column indicate significant differences ($p < 0.05$); identical letters indicate no significant difference ($p > 0.05$). Significance was determined by one-way ANOVA with Tukey's HSD post hoc test and confirmed by linear regression analysis and the Kruskal-Wallis non-parametric test.

lowest in the early postpartum period (30–40 d), with grade A/B COCs at 40.99%, maturation rate at 69.72%, and blastocyst rate at 32.59%. By 40–50 d, cleavage rate improved significantly (82.42%; $p < 0.05$), but other parameters remained suboptimal. Marked improvement was observed at 50–60 d, with grade A/B COCs reaching 54.45%, maturation rate 81.04%, and blastocyst rate 35.89% ($p < 0.05$ vs. 30–40 d). The highest values were achieved at 60–70 d, with blastocyst rate peaking at 39.71%.

3.5. Milk yield and oocyte developmental competence

Milk yield parameters—including daily milk production on the day of OPU, weekly average milk yield, and total 305-day yield—did not show statistically significant associations with oocyte developmental competence in Holstein cows ($p > 0.05$, Tables 5–7). Although cows in the highest production categories (e.g., 60–70 kg/day or 15–16 t/305 days) exhibited a numerical trend toward lower blastocyst rates, these

differences were not significant, suggesting that high lactational output alone does not necessarily impair oocyte quality. Consequently, within the production ranges evaluated, milk yield does not appear to be a limiting factor for donor selection in OPU-IVP programs, indicating that high-producing cows can be included as potential donors without compromising embryo developmental outcomes, which is valuable for maximizing genetic gain in commercial herds.

4. Discussion

The present study provides a comprehensive, field-based evaluation of donor factors affecting oocyte developmental competence in Holstein cows undergoing OPU-IVP. Analysis of data from 600 cows across three commercial farms allowed the identification of quantitative selection criteria that can be readily implemented in practice. Specifically, oocyte developmental competence was maximized in donors with a BCS of 2.75–3.25, age ≤80 months, parity ≤4, and 50–70 DIM, while milk

Table 5

Correlation analysis between milk yield on the day of OPU and the developmental potential of oocytes in Holstein multiparous cows.

Daily milk yield (kg)	Donors (n)	OPU sessions (n)	COC/donor (n)	Grade A/B COCs (%)	Maturation (%)	Fertilization (%)	Cleavage (%)	Blastocyst (%)
20–30	98	196	17.43	55.44 ± 1.48 ^a	81.93 ± 0.56 ^a	82.78 ± 2.09 ^a	82.89 ± 2.58 ^a	36.98 ± 1.65 ^a
30–40	108	216	16.04	55.37 ± 0.73 ^a	81.79 ± 0.86 ^a	79.54 ± 3.50 ^a	81.87 ± 1.82 ^a	38.84 ± 1.96 ^a
40–50	152	304	16.58	55.81 ± 1.67 ^a	81.28 ± 1.11 ^a	82.87 ± 2.73 ^a	83.49 ± 1.51 ^a	35.88 ± 1.52 ^a
50–60	143	286	16.63	54.19 ± 1.19 ^a	80.29 ± 1.49 ^a	80.40 ± 1.33 ^a	80.33 ± 3.84 ^a	32.09 ± 1.33 ^a
60–70	99	198	16.13	50.96 ± 0.96 ^a	78.01 ± 0.32 ^a	75.82 ± 1.28 ^a	77.72 ± 3.85 ^a	31.31 ± 2.38 ^a

Data were presented as means ± SEM. Different letters within the same column indicate significant differences ($p < 0.05$); identical letters indicate no significant difference ($p > 0.05$). Significance was determined by one-way ANOVA with Tukey's HSD post hoc test and confirmed by linear regression analysis and the Kruskal-Wallis non-parametric test.

Table 6

Correlation analysis between average weekly milk yield of donors and the developmental potential of oocytes in Holstein multiparous cows.

Weekly average milk yield (kg)	Donors (n)	OPU sessions (n)	COC/donor (n)	Grade A/B COCs (%)	Maturation (%)	Fertilization (%)	Cleavage (%)	Blastocyst (%)
20–30	90	180	16.27	53.52 ± 1.04 ^a	81.62 ± 1.82 ^a	80.46 ± 1.19 ^a	82.10 ± 1.39 ^a	37.36 ± 0.79 ^a
30–40	130	260	18.69	53.84 ± 0.49 ^a	79.93 ± 2.08 ^a	81.79 ± 0.90 ^a	81.69 ± 1.67 ^a	36.53 ± 0.82 ^a
40–50	143	286	16.98	53.41 ± 1.27 ^a	80.27 ± 2.57 ^a	80.78 ± 1.79 ^a	79.10 ± 1.25 ^a	35.28 ± 0.43 ^a
50–60	122	244	17.20	52.71 ± 1.18 ^a	80.64 ± 1.24 ^a	79.27 ± 1.65 ^a	78.59 ± 1.04 ^a	35.41 ± 0.73 ^a
60–70	115	230	16.51	49.34 ± 0.93 ^a	78.72 ± 1.49 ^a	76.64 ± 0.41 ^a	76.92 ± 0.63 ^a	34.63 ± 0.87 ^a

Data were presented as means ± SEM. Different letters within the same column indicate significant differences ($p < 0.05$); identical letters indicate no significant difference ($p > 0.05$). Significance was determined by one-way ANOVA with Tukey's HSD post hoc test and confirmed by linear regression analysis and the Kruskal-Wallis non-parametric test.

Table 7

Correlation analysis between the 305-day milk yield of donor cows and the developmental potential of oocytes in Holstein multiparous cows.

305-d milk yield (t)	Donors (n)	OPU sessions (n)	COC/donor (n)	Grade A/B COCs (%)	Maturation (%)	Fertilization (%)	Cleavage (%)	Blastocyst (%)
11–12	70	140	18.09	53.67 ± 0.71 ^a	82.51 ± 1.29 ^a	82.31 ± 0.95 ^a	82.35 ± 0.98 ^a	39.88 ± 0.49 ^a
12–13	141	282	17.25	53.03 ± 0.44 ^a	83.84 ± 2.50 ^a	79.71 ± 2.14 ^a	81.73 ± 0.87 ^a	39.99 ± 1.50 ^a
13–14	133	266	17.63	53.72 ± 0.20 ^a	80.41 ± 1.12 ^a	82.06 ± 0.48 ^a	79.92 ± 1.37 ^a	39.07 ± 3.07 ^a
14–15	131	262	17.53	53.58 ± 0.52 ^a	79.09 ± 1.19 ^a	78.40 ± 2.80 ^a	80.82 ± 1.62 ^a	38.27 ± 1.31 ^a
15–16	125	250	18.18	51.43 ± 0.67 ^a	77.77 ± 0.46 ^a	76.22 ± 0.29 ^a	77.94 ± 0.45 ^a	33.47 ± 1.14 ^a

Data were presented as means ± SEM. Different letters within the same column indicate significant differences ($p < 0.05$); identical letters indicate no significant difference ($p > 0.05$). Significance was determined by one-way ANOVA with Tukey's HSD post hoc test and confirmed by linear regression analysis and the Kruskal-Wallis non-parametric test.

yield within the ranges studied did not significantly influence oocyte quality or embryo development. The optimal BCS likely reflects a balanced metabolic and endocrine state that supports follicular development, whereas age and parity thresholds are consistent with the effects of reproductive aging on ovarian reserve and oocyte competence. Furthermore, the 50–70 DIM window corresponds to a period of postpartum uterine recovery and metabolic stabilization, which may enhance the intrafollicular environment. These findings provide a practical framework for selecting Holstein donors, enabling veterinarians and embryologists to improve the efficiency and predictability of OPU-IVP programs under commercial conditions.

The curvilinear relationship observed between BCS and oocyte developmental competence in this study confirms that both negative and positive energy balance can compromise reproductive function. BCS serves as a practical indicator of body energy reserves, and extreme values reflect metabolic states detrimental to folliculogenesis and oocyte quality [5]. Donors with low BCS (≤ 2.5) typically experience negative energy balance postpartum, characterized by elevated circulating non-esterified fatty acids and β -hydroxybutyrate, which have been shown to impair oocyte cytoplasmic maturation and increase oxidative stress within the follicle [5,18,19]. Conversely, overconditioned cows ($\text{BCS} \geq 3.75$) exhibit insulin resistance and chronic low-grade inflammation, leading to altered follicular fluid composition and compromised oocyte developmental programming [6]. These results indicate that a BCS range of 2.75–3.25 provides optimal oocyte quality, which is consistent with previous findings that cows with BCS between 3.00 and 3.50 maintain optimal health and reproductive function [5]. Collectively, these findings support the concept that extremes in BCS compromise oocyte cytoplasmic and nuclear maturation, likely through altered metabolic and endocrine environments. The relatively narrow optimal window highlights the importance of precision nutritional management of donor cows to maximize OPU-IVP efficiency.

Our findings indicate that oocyte developmental competence declines markedly in Holstein cows older than 80 months and beyond the fourth parity, corroborating well-established effects of reproductive aging in cattle [12,17]. The age-related reduction in oocyte quality is likely due to progressive depletion of the ovarian follicular reserve and accumulation of oxidative damage in mitochondrial DNA and other cellular components [15], increased oocyte aneuploidy, and mitochondrial dysfunction—hallmarks of reproductive aging in cattle. Parity,

although correlated with age, may exert additional effects through cumulative physiological stress associated with repeated gestation and lactation cycles. Notably, blastocyst rates remained stable through the first four parities but declined thereafter, suggesting that these initial lactations represent the prime reproductive lifespan for Holstein cows under intensive management. Together with the effects of BCS discussed previously, these results underscore the importance of prioritizing donors ≤ 80 months of age and ≤ 4 parities to maximize embryo production efficiency in commercial OPU-IVP programs.

The progressive improvement in oocyte quality observed from 30 to 70 DIM reflects the dynamic physiological recovery of postpartum Holstein cows. During the early postpartum period (30–40 d), incomplete uterine involution, disrupted ovarian follicular wave dynamics, and profound metabolic adaptations associated with lactation collectively create a suboptimal intrafollicular environment for oocyte maturation [20]. Developmental competence improves progressively as DIM advances, with optimal oocyte quality achieved at 50–70 DIM, consistent with reports indicating that complete uterine involution and restoration of normal ovarian cyclicity require approximately 40–50 days in well-managed dairy cows [21]. Importantly, substantial variation in oocyte quality persists even within the voluntary waiting period (30–70 d), suggesting that scheduling OPU procedures after 50 DIM can significantly enhance IVP outcomes without extending the calving-to-conception interval. These findings provide practical guidance for selecting postpartum donors to maximize embryo yield and overall OPU-IVP efficiency.

Importantly, this study found no significant correlation between milk yield and oocyte developmental competence within the production ranges evaluated. This observation challenges the conventional perception that high-producing cows are inherently less fertile [22]. A plausible explanation is that modern dairy cows, under optimal nutritional and management conditions, possess sufficient metabolic flexibility to allocate resources between lactation and reproduction without compromising oocyte quality [23]. Alternatively, the lack of statistical significance may reflect the relatively narrow milk yield range in the study population. Nevertheless, the observed numerical decline in blastocyst rates among the highest-yielding cows suggests the potential existence of a physiological threshold beyond which lactational demands may negatively affect oocyte competence. These findings indicate that, within practical production ranges, high-producing Holstein

cows can be considered suitable donors for OPU-IVP, thereby expanding the pool of potential donors for commercial embryo production programs.

The donor selection criteria established in this study—BCS 2.75–3.25, age ≤ 80 months, parity ≤ 4 , and DIM 50–70 d—provide a practical framework for optimizing OPU-IVP efficiency in commercial Holstein herds. These criteria are based on easily measurable phenotypic traits and can be readily implemented by farm personnel and veterinarians. Importantly, the lack of a significant effect of milk yield on oocyte competence broadens the pool of potential donors to include high-producing cows, thereby facilitating accelerated genetic improvement. This study has several limitations. First, the analysis was restricted to Holstein cows, and the applicability of these criteria to other breeds, particularly *Bos indicus* or crossbred cattle, remains to be determined. Second, although confounding factors were partially controlled by matching groups, residual effects of diet composition, genetic background, or other unmeasured variables cannot be entirely excluded. Third, direct measurements of follicular fluid metabolites or oocyte molecular markers were not performed, which could provide mechanistic insight into the observed associations. Future studies incorporating metabolomic and transcriptomic analyses may further refine donor selection strategies. In addition, while the numerical decline in blastocyst rates at extreme milk yields was not statistically significant, larger studies or those targeting ultra-high-producing cows (>70 kg/day) may clarify whether a biological threshold exists. In conclusion, this large-scale field study establishes quantitative donor selection criteria for Holstein cows undergoing OPU-IVP. Implementation of these guidelines can enhance the efficiency, predictability, and economic viability of commercial OPU-IVP programs, ultimately contributing to accelerated genetic progress in dairy herds.

5. Conclusion

This large-scale field study involving 600 Holstein cows across three commercial dairy farms establishes quantitative donor selection criteria for OPU-IVP programs. Optimal oocyte developmental competence is achieved in donors with a BCS of 2.75–3.25, age ≤ 80 months, parity ≤ 4 , and 50–70 DIM, while milk yield within the ranges studied does not significantly affect oocyte competence. These easily measurable phenotypic criteria provide a practical framework for improving the efficiency and predictability of bovine embryo production in commercial settings. Implementation of these guidelines can enhance genetic progress in dairy herds without extending the voluntary waiting period or excluding high-producing cows from the donor pool.

Data availability statement

The data involved in this study will be made available upon reasonable request to the corresponding author.

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CRediT authorship contribution statement

Heqiang Li: Conceptualization, Methodology, Software, Writing – original draft. **Baobao Zhao:** Conceptualization, Methodology, Software, Writing – original draft. **Jingya Wang:** Data curation, Validation, Writing – review & editing. **Xuexiao Song:** Data curation, Validation, Writing – review & editing. **Jieyu Wang:** Data curation, Validation, Writing – review & editing. **Yang Li:** Data curation, Validation, Writing – review & editing. **Zihan Xu:** Conceptualization, Validation, Writing –

review & editing. **Zheng Wang:** Conceptualization, Validation, Writing – review & editing. **Yaochang Wei:** Methodology, Resources, Supervision, Writing – review & editing. **Zhuangsheng Wang:** Methodology, Resources, Supervision, Writing – review & editing. **Yaping Jin:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Yongsheng Wang:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare that there are no conflicts of interest; financial; personal or other relationship with other people and organization.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.theriogenology.2026.118033>.

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