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Comparison of selected data acquisition models using on-farm production records on qualitative parameters of oocytes in dairy cows

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Abstract: Dairy cows enter the negative energy balance in the early post-partum period, which negatively affects milk yield, health status and reproduction. This study aimed to determine if milk yield (MY) and fat/protein (F/P) ratio could be used as reliable indicators for predicting the quality of aspirated oocytes and their further use for *in vitro* fertilisation. A secondary goal was to compare different periods before oocyte aspiration to determine which period had the most significant impact on the oocyte development in donor cows. All tested Holstein cows were at their first lactation (n = 68). The first ultrasound-guided follicular aspiration in donor cows was done on average at 58.7 days in milk (DIM). Usually, a second aspiration followed a week later if no problems were detected. In total, 102 aspirations were performed. Three model equations with different F/P ratio distributions and variants of MY production were tested in statistical evaluation. On average, 3.6 oocytes were retrieved per donor per aspiration, with a minimum of 1 and a maximum of 12. The maturation rate was 62.2%, with a high probability of reaching metaphase II (90.45%). The highest number of oocytes was obtained from low-yielding cows with a low F/P ratio in all evaluated periods ($P < 0.01$). The quality and expansion of cumulus-oocyte complexes were unaffected by the metabolic status. However, a tendency for better quality oocytes in donors with lower MY and F/P ratios was observed in all models. Our preliminary results showed that the prediction of oocyte quality in a homogeneous group of the first-lactation cows was related to the chosen data acquisition model. Data about milk yield and F/P ratio obtained from on-farm systems provide a valuable source of information for the selection of oocyte donors.

Keywords: milk yield; fat to protein ratio; ovum pick-up; maturation rate; lipid droplets

In order to meet the increased demand of the population for dairy products, the breeding of dairy cows has been focused on milk production in the last few decades (Fleming et al. 2018). Unfortunately, this way of breeding decreases reproduction, health, and longevity capabilities,

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as documented in many studies (Lucy 2001; Vacek et al. 2007; Leroy et al. 2008; Stadnik et al. 2017; Zavadilova et al. 2021; Kasna et al. 2022). Although in recent decades cattle breeding has also been aimed at the above-mentioned secondary functional traits (Pribyl et al. 2004), breeders are faced with a wide range of environmental effects negatively affecting the reproductive capabilities and fertility results of dairy cows, for example, heat stress of dairy cows as a result of global warming (Bezdicek et al. 2021).

From a breeding point of view, the time from calving to conception is one of the most critical periods of a cow's life. In order to achieve high breeding aims (i.e. high milk yield, reasonable reproductive parameters, health, longevity), it is necessary to breed cows on specific days post-partum, mainly after the voluntary waiting period (Caraviello et al. 2006). The early post-partum period is characterised by robust metabolic changes in the cow's body, and the majority of dairy cows enter the negative energy balance (NEB) stage already before calving; the highest level is reached approximately two to four weeks later (Stadnik et al. 2015) and may last approximately 8–10 weeks in total (Butler 2005). It is proven that NEB negatively affects milk production and health status, and leads to poor reproductive outcomes (Lopes et al. 2006).

Poor fertility in post-partum dairy cows is, among others, demonstrated by weak signs of heat and poor pregnancy rates behind which disturbed oocyte and follicular development stands (Roche et al. 2000). This fertility problem is very challenging for vets and farmers in the sense of prediction the best time for breeding the cow post-partum (Boudaoud 2023). To overcome this situation, it is recommended to use various techniques on a herd level that help to predict the optimal time for breeding (Das et al. 2023).

For that reason, it is useful to monitor cows' metabolic status and milk yield, as these data can be used to decide whether the cow will be bred successfully (Gabor et al. 2008). Data monitoring can be done in several ways. On-farm recording of milk-related data, e.g., milk yield (MY), protein and fat as well as fat to protein ratio (F/P), can provide quick and valuable information describing the metabolic status of cows and therefore it could serve as a prediction of fertility outcomes (Stadnik et al. 2022).

Published studies using MY and F/P farm data recorded in various time intervals after calving related this monitoring to various fertility indices: e.g., first service conception, days to first service, calving to conception interval at first service (Saranjam et al. 2020), calving to conception interval (Podpecan et al. 2008) and embryo yield after superovulation (Stadnik et al. 2022). In their review, Leroy et al. (2008) pointed out that metabolic changes directly affect oocyte quality. Interestingly, no studies are available describing an assumption of the effect of MY and F/P ratio obtained from on-farm systems on oocyte quality around the end of the voluntary waiting period, which might help farmers manage reproduction in their herds.

Therefore, this study aimed to determine if milk yield and F/P ratio recorded and evaluated in different periods after calving of dairy cows could be used as a reliable indicator for predicting the quality of aspirated oocytes and their further use for *in vitro* fertilisation. A secondary goal was to compare different periods before oocyte aspiration to determine which period had the most significant impact on the oocyte development in donor cows.

MATERIAL AND METHODS

Animals

This experiment was carried out in accordance with the Czech legislation for Protection of Animals against Cruelty (Act No. 246/1992) and with Directive 2010/63/EU on Protection of Animals Used for Scientific Purposes. The study was conducted in the production environment of a commercial dairy farm with Holstein cows in the Central Bohemian Region of the Czech Republic. All tested cows were at their first lactation, and they (n = 68) were all diagnosed as cycling (*corpus luteum* present on the ovary). Cows were milked twice daily, and the composition of TMR was adjusted to the daily milk yield of this group of dairy cows.

Oocyte retrieval – ovum pick-up

All cows in the experiment were at early lactation. The first ultrasound-guided follicular aspiration in donor cows was done on average at 58.7 days in milk (DIM) with a minimum of 36 DIM and a maxi-

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mum of 75 DIM. Usually, a second aspiration followed a week later if no problems were detected. In total, 102 aspirations were performed. Cows were treated with epidural anaesthesia (4 ml of 2% Lidocaine, Fatro, Italy), and afterwards, the rectum and vulva were cleaned. The ovaries were visualised with a 7 MHz convex probe attached to an ultrasound scanner MyLabOne VET (Esaote, Italy). An 18 G disposable hypodermic needle was fitted to a custom-made intravaginal probe holder, and –75 mm Hg pressure was applied using a vacuum aspiration pump. A 50 ml tube for the retrieval of cumulus-oocyte complexes (COCs) was held at 37 °C and filled with commercial oocyte retrieval media (OPU, Cornwall, UK). Immediately after each aspiration, the content of the 50 ml tube was filtered through an embryo filter, and COCs were searched under a stereomicroscope. Retrieved oocytes were transferred in a commercial holding medium (BO-IVM, Cornwall, UK) to the laboratory within 2 h after ovum pick-up.

In vitro maturation

Unless otherwise indicated, the chemicals were purchased from Sigma (Sigma-Aldrich, St. Louis, MO, USA) and plastics from Nunclon (Nunc, Roskilde, Denmark). *In vitro* maturation was performed as described in Benesova et al. 2017.

After arrival of COCs to the laboratory, these were thoroughly washed and subjected to *in vitro* maturation in Modified Parker Medium (MPM) supplemented with 20 mM sodium pyruvate, 50 IU/ml penicillin, 50 µg/ml streptomycin, 10% foetal bovine serum (FBS), serum gonadotropin and chorionic gonadotropin (P. G. 600, 15 IU/ml; Intervet, Boxmeer, The Netherlands) without a paraffin overlay in four-well dishes under a humidified atmosphere for 24 h at 39 °C with 5% CO_2. After 24 h, oocytes were denuded by gentle pipetting and fixed.

To determine cumulus cell expansion and evaluate quality, COCs or denuded oocytes were scanned by Axiocam 208 Colour (Zeiss, Oberkochen, Germany) before and after maturation.

COCs were classified according to the appearance of their cumulus cells (Table 1), and their expansion was evaluated. Subsequently, denuded oocytes were also classified and divided into three categories based on the structure of their ooplasm (Table 2).

Table 1. Classification of cumulus-oocyte complexes

Class	Number of cumulus layers
1	more than five layers
2	2–5 layers
3	one layer (also just partly surrounding)
4	no cumulus cells

Immunofluorescence and lipid staining

Oocytes were fixed in 4% paraformaldehyde for 50 min at room temperature. After washing in phosphate-buffered saline, the oocytes were incubated in 0.5% (v/v) TritonX-100 for 15 minutes. All subsequent steps were done in PBS supplemented with 0.3% (w/v) BSA and 0.05% (w/v) saponin. Oocytes were blocked with 2% normal goat serum (NGS; Millipore Biosciences; St. Charles, MO, USA) for 1 h and incubated with mouse anti-tubulin (T6793; Sigma-Aldrich, St. Louis, MO, USA) primary antibody overnight at 4 °C. After thorough washing, the oocytes were incubated with anti-mouse secondary antibody Alexa Fluor 488 (Invitrogen, Waltham, MA, USA) for 1 h at room temperature in the dark. For lipid visualisation, oocytes were subsequently stained with Nile Red (Invitrogen, Waltham, MA, USA) at a final concentration of 10 µg/ml in PBS supplemented with 0.4% BSA for 40 min, washed and mounted on glass slides using SlowFade™ Diamond Antifade Mountant with DAPI (Invitrogen, Waltham, MA, USA). The samples were examined using Leica TCS SP5 (Leica Microsystems AG, Wetzlar, Germany). The images were processed using Image J software.

The presence of a meiotic spindle and polar body was observed. Subsequently, oocytes were divided into groups: 0 = germinal vesicle (GV), 1 = germinal vesicle breakdown (GVBD) or metaphase I, 2 = metaphase II, 3 = degraded oocytes. The relative fluorescence of lipid content was measured, and lipid droplets were evaluated based on their localisation (1 = homogeneous and 2 = heterogeneous) and size (L = large, M = medium, and S = small). Oocytes were divided into groups based on the size of lipid

Table 2. Classification of oocytes

	Class	Morphology of oocyte
↓ Quality	1	compact, homogeneous, slightly granular
	2	granular, heterogeneous
	3	fragmented, heavily granular

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droplets: 1 = S, 2 = M, 3 = L, or on the combination of these lipid droplet sizes: 4 = S + M, 5 = S + L, 6 = M + L, 7 = all sizes were present.

Milk yield and fat/protein ratio

Daily milk yield (litres of milk), fat and protein content were recorded by Afifarm (v4.1, Afikim, Israel). The fat/protein ratio was subsequently calculated. The chosen periods of data acquisition were as follows: the data for Models 1, 2 and 3 (for detailed model information, please follow the Statistical analysis section) were gathered within a period of 25–35 DIM, at the time of ovum pick-up and in the period from calving to ovum pick-up, respectively.

Statistical analysis

The SAS v9.4 software (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis of results. The basic parameters of the dataset were calculated using UNIVARIATE and MEANS procedures. The normal distribution of observations applied to the main evaluation. The REG procedure, STEPWISE method, was used to choose the suitable model for the evaluation of observed indicators. Several model equations with different fat/protein ratio distributions and variants of milk yield were tested. The best model for evaluation was chosen according to the Akaike information criterion. The GLM procedure with the Tukey-Kramer test was used for the detailed evaluation. Model equations were as follows:

Model 1. The fixed effect of fat/protein ratio in 25–35 days of milk, the fixed effect of average milk yield in kg in 25–35 days of milk and regression on the day in milk (DIM) were used in the final model equation. The model equation was as follows:

$$y_{ijk} = \mu + RAO_i + LAO_j + b \times (DIM) + e_{ijk} \quad (1)$$

where:

y_{ijk} – values of dependent variables (number of aspirated COCs, quality of COCs, expansion of COCs, quality of oocytes, developmental stage of matured oocytes, maturation rate, probability of reaching metaphase II, intensity of lipids, size of lipid droplets, distribution of lipid droplets);

μ – the general value of a dependent variable,

RAO_i – fixed effect of fat/protein ratio in 25–35 days of milk (i = < 1.035, n = 122; i = > 1.035, n = 123);

LAO_j – fixed effect of average milk yield in 25–35 days of milk (j = < 26.01 kg, n = 75; j = 26.01–30.01 kg, n = 83; j = > 30.01 kg, n = 87);

$b \times$ (DIM) – linear regression on the day in milk at aspiration;

e_{ijk} – residual error.

Model 2. The fixed effect of fat/protein ratio on the day of oocyte aspiration, the fixed effect of average milk yield in kg on the day of the first oocyte aspiration and regression on the day in milk (DIM) were used in the final model equation. The model equation was as follows:

$$y_{ijk} = \mu + RAT_i + LAT_j + b \times (DIM) + e_{ijk} \quad (2)$$

where:

y_{ijk} – values of dependent variables (number of aspirated COCs, quality of COCs, expansion of COCs, quality of oocytes, developmental stage of matured oocytes, maturation rate, probability of reaching metaphase II, intensity of lipids, size of lipid droplets, distribution of lipid droplets);

μ – the general value of a dependent variable;

RAT_i – fixed effect fat/protein ratio on the day of oocyte aspiration (i = < 1.001, n = 51; i = > 1.001, n = 194);

LAT_j – fixed effect of milk yield on the day of oocyte aspiration (j = < 25.31 kg, n = 76; j = 25.31–30.21 kg, n = 81; j = > 30.21 kg, n = 88);

$b \times$ (DIM) – linear regression on the day in milk at aspiration;

e_{ijk} – residual error.

Model 3. The fixed effect of average fat/protein ratio from calving to the day of the first oocyte aspiration, the fixed effect of average milk yield in kg to the day of the first oocyte aspiration, and regression on the day in milk (DIM) were used in the final model equation. The model equation was as follows:

$$y_{ijk} = \mu + RAP_i + LAP_j + b \times (DIM) + e_{ijk} \quad (3)$$

where:

y_{ijk} – values of dependent variables (number of aspirated COCs, quality of COCs, expansion

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of COCs, quality of oocytes, developmental stage of matured oocytes, maturation rate, probability of reaching metaphase II, intensity of lipids, size of lipid droplets, distribution of lipid droplets);

μ – the general value of a dependent variable;

RAP_i – fixed effect of average fat/protein ratio from calving to the day of the first oocyte aspiration (i = < 1.064, n = 121; i = > 1.064, n = 124);

LAP_j – fixed effect of average milk yield from calving to the day of the first oocyte aspiration (j = < 24.77 kg, n = 76; j = 24.77–29.01 kg, n = 86; j = > 29.01 kg, n = 83);

$b \times (DIM)$ – linear regression on the day in milk at aspiration;

e_{ijk} – residual error.

The levels $P < 0.01$ and $P < 0.05$ were used for statistical significance evaluation.

RESULTS

In total, 245 oocytes were obtained from 68 cows. On average, 3.6 oocytes were obtained per donor per aspiration, with a minimum of 1 and a maximum of 12. The quality of COCs based on the classification in Table 1 was on average 2.33, while 43 COCs had more than five layers of cumulus cells and 56 had no cumulus cells. The quality of oocytes was as follows: 1 – 110; 2 – 60; 3 – 35. The maturation rate was 62.2% with a high probability of reaching metaphase II (90.45%). The effects in the model equation were mostly significant, and the models explained 4.6 to 15.5% of variability.

Model 1: Data obtained in a period of 25–35 DIM

Table 3 shows the effect of low and high F/P ratios measured in milk during a period of 25–35 DIM on evaluated parameters of oocyte quality. Only the number of aspirated COCs was significantly influenced by the F/P ratio when more oocytes were aspirated in cows with low F/P ratio compared to those with high F/P ratio ($P < 0.01$).

Average daily milk yield during 25–35 DIM significantly affected the number of aspirated oocytes (Table 3). More oocytes were aspirated from cows with low compared to medium and high ($P < 0.01$)

Table 3. Model 1 – the effect of F/P ratio and milk yield during a period of 25–35 DIM on quantity and quality of aspirated oocytes calculated by GLM procedure (LSM ± SE)

	Number of aspirated COCs (n)	Quality of COCs[1]	Expansion of COCs[2]	Quality of oocytes[3]	Developmental stage of matured oocytes[4]	Maturation rate (% of metaphase II)	Probability of reaching metaphase II (%)	Relative fluorescence intensity of lipids (%)	Size of lipid droplets[5]	Distribution of lipid droplets[6]
F/P										
< 1.035	4.15 ± 0.23^{A}	2.31 ± 0.11	47.00 ± 5.40	1.72 ± 0.07	1.76 ± 0.07	65.47 ± 3.12	93.15 ± 2.92	64.70 ± 1.84	3.71 ± 0.18	1.22 ± 0.04
> 1.035	2.70 ± 0.24^{B}	2.36 ± 0.11	48.62 ± 5.69	1.55 ± 0.08	1.86 ± 0.08	58.58 ± 3.20	87.49 ± 3.00	69.82 ± 1.87	3.81 ± 0.19	1.19 ± 0.04
Milk yield										
< 26.01	4.46 ± 0.30^{A}	2.38 ± 0.14	45.96 ± 7.14	1.73 ± 0.09	1.70 ± 0.10	57.97 ± 3.98	87.43 ± 3.73	60.88 ± 2.33^{Aa}	3.96 ± 0.23	1.19 ± 0.05
26.01–30.01	2.83 ± 0.29^{B}	2.40 ± 0.13	46.12 ± 6.64	1.69 ± 0.10	1.88 ± 0.09	60.70 ± 3.90	89.33 ± 3.65	72.37 ± 2.31^{B}	3.60 ± 0.23	1.25 ± 0.05
> 30.1	2.97 ± 0.28^{B}	2.22 ± 0.13	51.35 ± 6.61	1.48 ± 0.09	1.85 ± 0.09	67.42 ± 3.82	94.19 ± 3.58	68.53 ± 2.22^{b}	3.71 ± 0.22	1.18 ± 0.05

COCs = cumulus-oocyte complexes; F/P = fat to protein ratio in milk; LSM = least square means; SE = standard error of least square means

[1]Explained in Table 1; [2]expansion was evaluated as a binary parameter yes = 100/no = 0; [3]explained in Table 2; [4]0 = germinal vesicle,1= metaphase I, germinal vesicle breakdown, 2= metaphase II, 3= degraded; [5]small (S) = 1, medium (M) = 2, large (L) = 3, or combination of these lipid droplet sizes: S + M = 4, S + L = 5, M + L = 6, all sizes present = 7; [6]classified as homogeneous = 1 and heterogeneous = 2

[A,B]Different letters in columns indicate statistical significance, $P < 0.01$; [a,b]different letters in columns indicate statistical significance, $P < 0.05$

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average daily milk yield. The maturation rate (percentage of oocytes reaching the metaphase II stage after *in vitro* cultivation) had a tendency to be higher in cows with high milk yield compared to those with low milk yield. The fluorescence signal intensity of lipid droplets in matured oocytes was higher in cows assigned to higher and medium groups than in the low group ($P < 0.05$, resp. $P < 0.01$).

Model 2: Data obtained on the day of oocyte aspiration

The influence of the F/P ratio measured on the day of oocyte aspiration on oocyte quality parameters is presented in Table 4. When the F/P ratio was low, the number of aspirated COCs was significantly lower compared to the high F/P ratio ($P < 0.05$). The quality of aspirated COCs evaluated before *in vitro* cultivation was better in low F/P cows compared to high F/P cows ($P < 0.01$). The maturation rate of oocytes retrieved from cows with low F/P ratio was significantly higher compared to cows with high F/P ratio ($P < 0.01$). There was no significant effect of F/P on the rest of the investigated parameters.

The milk yield on the day of oocyte retrieval significantly influenced the number of aspirated COCs (Table 4). Ovum pick-up from cows in the low milk yield group yielded significantly more COCs compared to cows in the medium and high groups ($P < 0.05$). Larger cytoplasmic lipid droplets were found in oocytes aspirated from cows in the medium compared to the low milk yield group ($P < 0.05$).

Model 3: Data obtained in the period from calving to oocyte aspiration

The effect of the F/P ratio measured in the period from calving to oocyte aspiration is shown in Table 5. As with Model 1, only the number of aspirated oocytes differed between cows with low and high F/P ratio, favouring the low group ($P < 0.01$).

The average milk yield significantly influenced the number of aspirated COCs, maturation rate, and intensity of lipids in matured oocytes (Table 5). The highest number of COCs was ob-

Table 4. Model 2 – the effect of F/P ratio and milk yield at the day of the first oocyte aspiration on quantity and quality of aspirated oocytes calculated by GLM procedure (LSM ± SE)

	Number of aspirated COCs (n)	Quality of COCs[1]	Expansion of COCs[2]	Quality of oocytes[3]	Developmental stage of matured oocytes[4]	Maturation rate (% of metaphase II)	Probability of reaching metaphase II (%)	Relative fluorescence intensity of lipids (%)	Size of lipid droplets[5]	Distribution of lipid droplets[6]
F/P										
< 1.001	2.96 ± 0.25[a]	2.11 ± 0.11[A]	47.25 ± 5.43	1.60 ± 0.08	1.73 ± 0.08	68.34 ± 3.23[A]	93.19 ± 3.09	68.07 ± 2.03	3.66 ± 0.19	1.22 ± 0.04
> 1.001	3.86 ± 0.25[b]	2.54 ± 0.11[B]	48.76 ± 5.87	1.68 ± 0.08	1.88 ± 0.08	56.32 ± 3.12[B]	87.81 ± 2.99	66.46 ± 1.93	3.87 ± 0.19	1.20 ± 0.04
Milk yield										
< 25.31	4.17 ± 0.31[a]	2.30 ± 0.14	50.58 ± 7.00	1.66 ± 0.10	1.72 ± 0.10	56.84 ± 3.92	87.69 ± 3.75	64.92 ± 2.52	3.39 ± 0.23[a]	1.28 ± 0.05
25.31–30.21	2.96 ± 0.31[b]	2.35 ± 0.14	47.72 ± 7.23	1.66 ± 0.10	1.79 ± 0.10	61.71 ± 4.06	90.11 ± 3.89	67.73 ± 2.51	4.22 ± 0.24[b]	1.17 ± 0.05
> 30.21	3.10 ± 0.29[b]	2.34 ± 0.13	45.72 ± 6.52	1.58 ± 0.09	1.92 ± 0.09	68.45 ± 3.71	93.71 ± 3.55	69.15 ± 2.28	3.68 ± 0.22	1.18 ± 0.05

COCs = cumulus-oocyte complexes; F/P = fat to protein ratio in milk; LSM = least squares means; SE = standard error of least squares means

[1]Explained in Table 1; [2]expansion was evaluated as a binary parameter yes = 100/no = 0; [3]explained in Table 2; [4]0 = germinal vesicle,1= metaphase I, germinal vesicle breakdown, 2= metaphase II, 3= degraded; [5]small (S) = 1, medium (M) = 2, large (L) = 3, or combination of these lipid droplet sizes: S + M = 4, S + L = 5, M + L = 6, all sizes present = 7; [6]classified as homogeneous = 1 and heterogeneous = 2

[A,B]Different letters in columns indicate statistical significance, $P < 0.01$; [a,b]different letters in columns indicate statistical significance, $P < 0.05$

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Table 5. Model 3 – the effect of F/P ratio and milk yield during the period from calving to aspiration on quantity and quality of aspirated oocytes calculated by GLM procedure (LSM ± SE)

	Number of aspirated COCs (*n*)	Quality of COCs[1]	Expansion of COCs[2]	Quality of oocytes[3]	Developmental stage of matured oocytes[4]	Maturation rate (% of metaphase II)	Probability of reaching metaphase II (%)	Relative fluorescence intensity of lipids (%)	Size of lipid droplets[5]	Distribution of lipid droplets[6]
F/P										
< 1.064	4.14 ± 0.23^{A}	2.35 ± 0.11	47.65 ± 5.43	1.71 ± 0.07	1.77 ± 0.08	64.98 ± 3.13	92.52 ± 2.95	65.86 ± 1.89	3.65 ± 0.18	1.20 ± 0.04
> 1.064	2.68 ± 0.24^{B}	2.32 ± 0.11	47.39 ± 5.64	1.55 ± 0.08	1.85 ± 0.08	59.45 ± 3.21	88.37 ± 3.02	68.82 ± 1.92	3.86 ± 0.19	1.22 ± 0.04
Milk yield										
< 24.77	4.24 ± 0.30^{Aa}	2.52 ± 0.14	41.08 ± 7.07	1.72 ± 0.09	1.71 ± 0.09	54.65 ± 3.89^{a}	86.00 ± 3.67	62.26 ± 2.33^{a}	3.83 ± 0.23	1.28 ± 0.05
24.77–29.01	3.16 ± 0.28^{b}	2.23 ± 0.13	53.07 ± 6.50	1.66 ± 0.09	1.91 ± 0.09	63.65 ± 3.82	91.49 ± 3.60	70.74 ± 2.32^{b}	3.82 ± 0.22	1.13 ± 0.05
> 29.1	2.82 ± 0.29^{B}	2.25 ± 0.13	48.41 ± 6.81	1.52 ± 0.09	1.83 ± 0.10	68.36 ± 3.96^{b}	93.85 ± 3.73	69.01 ± 2.35	3.60 ± 0.23	1.22 ± 0.05

COCs = cumulus-oocyte complexes; F/P = fat to protein ratio in milk; LSM = least squares means; SE = standard error of least squares means
[1]Explained in Table 1; [2]expansion was evaluated as a binary parameter yes = 100/no = 0; [3]explained in Table 2; [4]0 = germinal vesicle, 1 = metaphase I, germinal vesicle breakdown, 2 = metaphase II, 3 = degraded; [5]small (S) = 1, medium (M) = 2, large (L) = 3, or combination of these lipid droplet sizes: S + M = 4, S + L = 5, M + L = 6, all sizes present = 7; [6]classified as homogeneous = 1 and heterogeneous = 2
^{A,B}Different letters in columns indicate statistical significance, $P < 0.01$; ^{a,b}different letters in columns indicate statistical significance, $P < 0.05$

tained from cows with low milk yield compared to the group of cows with medium ($P < 0.05$) and high ($P < 0.01$) milk yield. The maturation rate of COCs was higher in cows from the high milk yield group compared to the low milk yield group ($P < 0.05$), and the fluorescence intensity of lipid droplets of matured oocytes was higher in the medium group compared to the low group ($P < 0.05$).

Comparison of the models

As it is noticeable from the presented tables, trends for the quantity of oocytes were similar among the models. Most oocytes were retrieved from low-yielding cows with a low F/P ratio in all evaluated periods ($P < 0.01$). Quality and expansion of COCs were unaffected by the metabolic status of donor cows. However, a tendency for better quality oocytes in donors with lower MY and F/P ratios was observed in all models. The best results for maturation rate (tendency in Model 1, $P < 0.01$ in Models 2 and 3) and a tendency for a higher probability of reaching metaphase II across models were achieved by low F/P ratio and high milk yields, which applies to the cows that would probably be in the worse negative energy balance, and therefore in the worse metabolic status. Fluorescence intensity of lipids, size and distribution of lipid droplets did not show any tendencies for F/P ratio, but significant results were achieved for milk yield groups. However, trends for the size and distribution of lipid droplets varied across models. A significant trend was observed for the fluorescence intensity of lipid droplets, with the lowest values achieved by the low milk yield group.

Observed tendencies could become significant with more animals in the test. Based on the results, we recommend using Model 3 for the selection of the donor cows because it fully covers the donor's metabolic status between calving and aspiration. On the other hand, Model 2 only uses data from a single day, which could make the selection process easier, and specifically in regards to F/P ratio significant differences in oocyte quality were detected. Ultimately, all three models could be suitable for donor cow selection as they show similar trends and tendencies for evaluated parameters.

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DISCUSSION

This study aimed to describe the effect of three various data acquisition models using on-farm production records on qualitative parameters of oocytes obtained via ovum pick-up 36–75 DIM and matured *in vitro*. With this approach, we aimed to shed light on a better selection of cows for insemination or embryo transfer or *in vitro* embryo production. The data for models 1, 2, and 3 were gathered within 25–35 DIM, at the time of ovum pick-up, and in the period from calving to ovum pick-up, respectively.

Our preliminary results obtained from the Holstein cows at first lactation showed that the metabolic status of a cow (measured via F/P ratio and milk yield in kg/day) influenced the quantity and quality of oocytes harvested in the early post-partum period. Matoba et al. (2012) stated in their study that oocyte quality (morphological assessment, ability to undergo fertilisation and develop to the blastocyst stage) was not affected by the post-partum metabolic status. However, they used cows at third lactation (3.0 ± 0.4) and evaluated the effect between periods of 0–42 and 42–80 days post partum. Roth et al. (2008) did not find any metabolic effect on the oocytes of cows at their second and later lactations when they compared the breeding period (60–95 days post partum) vs. middle lactation (120–225 days post partum). On the other hand, Kendrick et al. (1999) described impaired oocyte morphology at approximately 100 days compared to 30 days post partum. From the above-mentioned studies it seems that stages of lactation affected oocyte quality rather than lactation number.

In our models, we used three stages of lactation in the post-partum period of metabolic and milk yield data acquisition (Models 1–3) to find out which model is better for the prediction of oocyte quality close to the time of breeding. In general, our three models show similar effects of F/P ratio and milk yield on oocyte quality. Our results show that when metabolic and milk yield data are gathered within periods of 25–35 DIM (Model 1) and from calving to oocyte aspiration (Model 3), the predictive value is very similar. Major hormonal and inflammatory changes occur at the onset of lactation, and these changes coincide with the sensitivity window of oocytes and follicles in development (Leroy et al. 2011). This chain of events may lead to significantly affected oocyte and granulosa cell function, with an adverse impact on reproductive performance at the moment of breeding (Leroy et al. 2022). On the other hand, when data were recorded at the time of oocyte aspiration on 36–75 DIM (Model 2), we could see a higher influence of the F/P ratio and a lower influence of milk yield. Variability in our prediction of oocyte quality outcomes may correspond to the adaptation or maladaptation of cows to metabolic stress induced by early lactation (Pascottini et al. 2022).

Therefore, the selection of lactating cows suitable for oocyte aspiration should be performed carefully as the oocyte quality is a key factor driving the final blastocyst rate in *in vitro* embryo production (IVP) (Lonergan and Fair 2016). With the results of our study, monitoring metabolic status (F/P ratio) and milk yield may provide an insight into this selection process. Nevertheless, we are aware that other indicators of the metabolic and health status in transition period (Leblanc 2010), closer monitoring of the resumption of ovarian activity (Shrestha et al. 2004) and body condition score (Bezdicek et al. 2020) may help to select cows as suitable donors of oocytes for IVP in later stages of lactation.

CONCLUSION

Our preliminary results showed that the prediction of oocyte quality in a homogeneous group (same housing, feed ratio, management, parity) of the first-lactation Holstein cows is related to the chosen data acquisition model, and fat to protein ratio along with milk yield obtained from on-farm systems provides a valuable source of information for the selection of oocyte donors. Cows with low milk yield had a higher quantity of aspirated oocytes but of worse quality. The F/P ratio significantly affected the number of aspirated oocytes, while effects on quality were mostly insignificant. However, similar tendencies and trends were observed, which could become significant with an increased number of tested animals, as stable trends were observed for most parameters. In order to achieve a more precise decision model, it would be beneficial to incorporate more farm and health data about tested cows.

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Conflict of interest

The authors declare no conflict of interest.

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