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THE INFLUENCE OF INDIVIDUAL FEATURES AND THE BREED OF DONOR HEIFERS ON THE EFFICIENCY OF OOCYTE RETRIEVAL BY OVUM PICK-UP

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Abstract

The oocytes' retrieval from lived cows (Ovum Pick-Up, OPU) is the most important element in the system of *in vitro* production (IVP) of cow embryos. In this regard, increasing the efficiency of OPU is a key factor for the widespread implementation of IVP technology in cattle breeding. The present work shows for the first time the possibility of obtaining IVP embryos from OPU oocytes obtained from a local breed of Yaroslavl cows. In Yaroslavl breed comparing to Simmental breed, the variability of the number of follicles was observed both between individual animals and between sessions in the same animals. We did not observe significant differences in the recovery rate and qualitative characteristics of the obtained cumulus-oocyte complexes, which allows using standardized protocols for donor heifers of both Simmental and Yaroslavl breeds. The aim of our work was to study the influence of individual features and breed characteristics of donor heifers on the efficiency of oocyte production by OPU. The studies were carried out in the laboratory of experimental embryology in 2020-2022 on mature clinically healthy heifers of the Simmental and Yaroslavl breeds aged of 17 to 36 months. Oocytes were obtained by transvaginal ultrasound-controlled puncture of follicles with an interval between sessions of 7 days according to the methodical guidelines using systems Bovine OPU (Minitube GmbH, Germany). In the first experiment, the efficiency of OPU was studied in individual donor heifers of the Simmental (n = 7; 50 sessions) and Yaroslavl breeds (n = 5; 25 sessions). In the second experiment, a comparative study of the OPU efficiency in donor heifers of the Simmental (n = 6; 12 sessions) and Yaroslavl breeds (n = 5; 25 sessions) was carried out using OPU parameters optimized for animals of the Simmental breed. The OPU efficiency was evaluated using the following criteria: the number of ultrasound-visible follicles, the number of retrieved cumulus-oocyte complexes (COCs), the degree of oocyte retrieval, the ratio of oocytes suitable for production of IVP embryos from the total number of derived oocytes. We observed high variability of the average number of ultrasound-visible follicles among individual donor heifers, both of Simmental (4.71-11.50 follicles; Cv = 47.9 %) and Yaroslavl breeds (5.80-9.80 follicles, Cv = 32.0 %), while the differences between some donors within breeds were highly significant ($p \leq 0.001$). Differences in the number of ultrasound-visible follicles led to differences in the number of derived COCs among individual donors, both of Simmental (2.33-5.17 COCs) and Yaroslavl breeds (3.60-6.00 COCs). Comparative studies of donor heifers of the Simmental and Yaroslavl breeds did not show significant differences in the average number of ultrasound-visible follicles (7.33±0.62 vs. 6.96±0.45), the number of obtained oocytes (4.17±0.69 vs. 3.36±0.41) and the number of suitable oocytes (3.08±0.60 vs. 2.52±0.29). Thus, a high variability in the average number of ultrasound-visible follicles and the number of obtained oocytes between individual donor heifers of the Simmental and Yaroslavl breeds was established. Considering the high positive correlation between the number of aspirated follicles and the number of retrieved oocytes (r = 0.97 and 0.72 for donor heifers of the Simmental and Yaroslavl breeds, respectively), it is advisable to select animals characterized by a large number of ultrasound-visible follicles to increase the efficiency of OPU. The absence of significant differences related to quantitative and qualitative characteristics of

the retrieved oocytes allows us to recommend the OPU parameters optimized for donor heifers of the Simmental breed to be applied in heifers of the Yaroslavl breed without a noticeable loss of efficiency.

Keywords: cattle, assisted reproductive technologies, OPU, in vitro embryo production

Currently, assisted reproductive technologies (ART) are the most important element in the genetic improvement of farm animals. The term ART describes procedures in which reproductive cycles, gametes or embryos are manipulated [1]. The relevance of the ART development is primarily due to the expected acceleration of selection and, as a result, genetic progress [2]. In addition, ARTs serve as an integral element in the development of genomic editing technologies [1-3].

In cattle, due to long generation interval and relatively low fecundity, ARTs are of particular relevance. ARTs used in cattle breeding include artificial insemination (AI), cryopreservation of semen and embryos, synchronization of the sexual cycle, multiple ovulation and embryo transfer (MOET), ovum pick-up (OPU) and in vitro production of embryos (IVP), semen sexing, embryo sexing, nuclear transfer (NT) [1].

The first widely used ART in cattle was the AI technology [4] which received mass adoption with the discovery of capacity for further viability of gametes after cryopreservation [5]. AI technology has enabled breeders and practitioners to better exploit the genetic potential of outstanding sires. For females, this problem was initially solved by the development and implementation of the MOET technology [6]. Classical MOET technology includes superovulation of genetically best donor cows and their artificial insemination followed by washing out of embryo, usually on days 6-7 after insemination. The resulting embryos are transplanted to recipients with a synchronized sexual cycle immediately after washing or after freezing-thawing. Embryos obtained by the MOET technology are defined as in vitro derived (IVD). Despite the advantages of MOET technology, the main factor limiting its widespread use, remains the need for hormonal treatment to induce superovulation. The lack of response to hormonal stimulation in some donors, the decrease in the effectiveness of superovulation with each subsequent hormonal treatment, the need for a break of several months between hormonal treatments, as well as high hormone costs significantly increase the cost of the resulting embryos.

An alternative to MOET was the development of IVP technology, the essence of which is to collect cow oocytes from the ovaries by aspiration of follicles, oocyte maturation, fertilization and in vitro development of embryos to the stages of late morula or blastocyst [7]. In combination with OPU [8], IVP technology is increasingly used in cattle breeding. The advantages of OPU/IVP procedure compared to MOET are evidenced by the progressive increase in the number of bovine embryos produced by IVP despite a decrease in the number of embryos derived from the classic MOET procedure. Thus, from 2000 to 2020, the number of IVD embryos worldwide decreased from 664,220 to 361,728, while the number of IVP embryos increased from 139,372 to 1,156,422 [9, 10].

An increase in the effectiveness of OPU was a decisive factor for the widespread of IVP technology. In this regard, studies have been carried out to identify factors affecting OPU effectiveness and optimization. The factors that affect the OPU performance are the type of aspiration needle used [11-13] and vacuum pressure [12, 14, 15], intervals between OPU sessions [16, 17], and whether hormonal synchronization of the sexual cycle [18-20] and superstimulation [21, 22] were applied. Individual features of donors, including breed [23, 24], age [25, 26], stage of the sexual cycle and individual response [27], climatic conditions [28, 29], nutritional factors, and operator experience also affect the OPU performance. The

potential of the resulting OPU oocytes to be fertilized and further developed is affected by the conditions of maturation, fertilization, and in vitro cultivation (30).

Previously, we determined the optimal type of aspiration needle, vacuum pressure [13] and the frequency of sessions in Simmental donor heifers [17]. However, the individual influence of donor heifers on the OPU performance has not been studied. In addition, it is necessary to evaluate the effectiveness of the methodology for conducting OPU, developed for donor heifers of the Simmental breed, on other cattle breeds.

In the presented work, for the first time, the possibility and efficiency of obtaining OPU oocytes in Yaroslavl cows is shown. In the Yaroslavl breed, compared to the Simmental breed, the variability in the number of follicles has been revealed both between animals and between sessions when the same animal is subjected to the procedure. We did not find statistically significant differences in the degree of isolation and qualitative parameters of cumulus-oocyte complexes (COC) which allows us to use standardized protocols for donor heifers of the Simmental and Yaroslavl breeds.

The purpose of this work was to investigate the influence of individual and breed traits of the Simmental and Yaroslavl donor heifers on the effectiveness of OPU oocyte production.

Materials and methods. The studies were carried out at the Ernst Federal Research Center for Animal Husbandry (Ernst FRC VIZh) in 2020-2022 on sexually mature clinically healthy Simmental and Yaroslavl heifers (*Bos taurus taurus*) aged from 17 to 36 months. Animals were fed diets balanced in terms of energy, nutrients and biologically active substances in accordance with the norms [31]. Oocytes were retrieved by transvaginal sonographic-assisted follicle puncture in accordance with the guidelines [32] using the Bovine OPU system (Minitube GmbH, Germany) completed with an ultrasound scanner ProSound 2 (Hitachi Aloka Medical, Ltd., Japan), 5 MHz ultrasonic sector probe (Aloka UST-9111-5, 5 MHz/90°/14 mm) with holder and vacuum pump.

In the first experiment, we studied the influence of the individual traits of the Simmental donor heifers ($n = 7$, 50 sessions) and Yaroslavl donor heifers ($n = 5$, 25 sessions) on the OPU effectiveness. Puncture of all ultrasound-visible follicles was performed with a 7-day interval using a 1.2×75 mm needle (18G×3″, long cut) at a vacuum pressure of 80 mmHg (Simmental breed) and 90 mmHg (Yaroslavl breed).

In the second experiment, the OPU effectiveness was evaluated depending on the breed characteristics of the Simmental donor heifers ($n = 6$, 12 sessions) and Yaroslavl donor heifers ($n = 5$, 25 sessions). All ultrasound-visible follicles were punctured within one time period using OPU parameters optimized for Simmental animals: aspiration needle 1.2×75 mm (18G×3″, long cut), vacuum pressure 90 mmHg, interval between sessions 7 days.

The criteria for evaluating the OPU effectiveness were the number of follicles visible by ultrasound, the number of retrieved oocytes in the COC, the degree of COC picking up, percentage of the COCs potentially suitable for IVP out of the total number of retrieved COCs. COCs were considered eligible if there were no obvious signs of cytoplasmic abnormalities in oocytes (e.g., degeneration, lysis, contraction, irregular shape), with the exception of mature COCs. Oocytes with homogeneous ooplasm, completely or partially devoid of cumulus cells, were considered suitable.

The resulting digital data were processed using the variation statistics methods in the Microsoft Excel program. In the experimental groups, the arithmetic mean values (M), standard deviations (σ), standard errors of the means ($\pm$SEM) were calculated. To assess the variability of the studied parameters, the coefficient of variation (Cv) was calculated. Statistical significance of differences

in arithmetic mean values was determined using Student's *t*-test. To compare two samples in terms of relative values, expressed as a percentage, Fisher's test with an angular transformation (φ-test) was used. The values were considered highly significant at $p \le 0.001$ and significant at $p \le 0.01$ and $p \le 0.05$.

Results. Significant differences were found in the number of ultrasound-visible follicles between individuals of both Simmental and Yaroslavl breeds (Table 1).

1. Number of ultrasound-visible follicles in donor heifers (*Bos taurus taurus*) Simmental and Yaroslavl breeds (the Ernst Federal Research Center for Animal Husbandry, Dubrovitsy, 2020-2022)

Breed	ID	Number of sessions	Number of follicles (*M*±SEM)	*Cv*, %
Simmental	3501	7	11.14±1.40[1, 5]	33.3
	3507	12	11.50±1.55[2, 6]	46.8
	3579	6	10.33±2.51[4]	59.6
	7019	6	9.33±0.33[3, 7]	8.7
	2480	9	7.78±0.57[1]	22.1
	2547	3	5.67±0.88[5, 6, 7]	27.0
	2581	7	4.71±0.46[1, 2, 3, 4]	26.6
	On average	50	9.08±0.61	47.9
Yaroslavl	451	5	9.80±1.16[1, 2, 3, 4]	26.4
	461	5	6.00±0.89[1]	33.3
	1884	5	5.80±0.49[2]	18.9
	1885	5	6.60±0.51[3]	17.3
	1890	5	6.60±0.81[4]	27.5
	On average	25	6.96±0.45	32.0

N o t e. ID — identification number of the heifer.
Differences between Simmental donor heifers marked with the same superscript numbers are statistically significant at [1, 2, 3]$p \le 0.001$, [5, 6, 7]$p \le 0.01$, and [4]$p \le 0.05$, between Yaroslavl donors heifers at [1, 2, 3, 4]$p \le 0.05$.

The average values of this indicator in Simmental donor heifers ranged from 4.71 to 11.50 follicles ($Cv = 47.9\%$), the differences between some donors were highly significant ($p \le 0.001$). The *Cv* values for the number of ultrasound-visible follicles in individual donors in different sessions were 8.7-59.6%, while there was a tendency for greater variability in heifers with a higher average value of the number of ultrasound-visible follicles ($r = 0.45$) . In Yaroslavl heifers compared to the Simmental heifers, there was a lower variability in the number of ultrasound-visible follicles both between heifers (5.80-9.80 follicles, $Cv = 32.0\%$), and in individual heifer between sessions ($Cv = 17.3$-33.3%).

The degree of COC extraction varied for Simmental heifers from 41.2 to 51.4%, for Yaroslavl heifers from 36.4 to 75.9%. As is known that a higher vacuum pressure during follicle puncture increases the COC extraction [12, 13], therefore, a higher rate in the Yaroslavl heifers may be due to the use of a higher vacuum pressure (90 mmHg) compared to Simmental heifers (80 mmHg).

A high positive correlation was noted between the number of punctured follicles and the number of obtained COCs in both Simmental heifers ($r = 0.97$, $p \le 0.01$) and Yaroslavl heifers ($r = 0.72$, $p \le 0.05$). The number of COCs retrieved from one donor per session averaged from 2.33 to 5.17 for Simmental heifers (with individual differences between donors being statistically significant, $p \le 0.001$) and from 3.60 to 6.00 for the Yaroslavl heifers (Table 2).

2. Number of cumulus-oocyte complexes (COCs) **obtained from donor Simmental and Yaroslavl heifers (*Bos taurus taurus*)** (the Ernst Federal Research Center for Animal Husbandry, Dubrovitsy, 2020-2022)

Breed	ID	Number of sessions	Number of COCs (*M*±SEM)	Suitable COCs, %
Simmental	3501	7	5.00±0.76[1]	77.14
	3507	12	5.17±0.93[2]	69.35[1]
	3579	6	5.00±1.03	70.00[2]
	7019	6	4.33±0.56	92.31[1, 2, 3]
	2480	9	4.00±0.58	80.56
	2547	3	2.33±0.88	57.14[3]
	2581	7	2.57±0.30[1, 2]	83.33

Continued Table 2

Yaroslavl	451	5	6.00±1.67	83.33[3]
	461	5	3.60±0.75	72.22[2]
	1884	5	4.40±0.40	72.73[1]
	1885	5	4.20±0.73	95.24[1. 2. 3]
	1890	5	2.40±0.68	83.33

N o t e. ID — identification number of the heifer.
Differences between Simmental donor heifers marked with the same superscript numbers are statistically significant on the number of COCs at $^{1,\ 2}p \le 0.05$, on suitable COC proportion at $^{1}p \le 0.01$, $^{2,\ 3}p \le 0.05$; between Yaroslavl donor heifers on suitable COC proportion at $^{1}p \le 0.01$, $^{2,\ 3}p \le 0.05$.

In the first experiment, the number of obtained COCs was not compared between the two breeds studied, since the time periods of manipulations with Simmental heifers and the Yaroslavl heifers differed. In addition, different vacuum pressures were used to aspirate follicles, which, as shown in numerous studies, affects the degree of oocyte retrieval, and hence the number of COCs obtained [12, 13, 15].

A high individual variability in the number of ultrasound-visible follicles and the number of received COCs was noted in a number of studies [24, 27]. In aboriginal Podolsk cattle, the number of follicles varied from 2.9 to 9.3, the number of COCs varied from 0.5 to 6.8 [24]; in Black-and-White cattle, the number of COCs was 1.6-9.2 [27].

Comparative studies conducted in the second experiment did not show statistically significant differences in the average number of ultrasound-visible follicles in the donor Simmental and Yaroslavl heifers (7.33±0.62 and 6.96±0.45, respectively). The values of this indicator we obtained generally agree with the results of other authors. Thus, in highly productive dairy Holsteinized Black-and-White cows, the average number of visualized follicles per donor was 3.88 [33], 4.81 [12] and 6.10 [34]. On Podolsk cattle, on average, 5.00 follicles were found in one donor [24].

We also did not observe significant differences in the degree of COC extraction between the Simmental and Yaroslavl heifers, 67.05 and 59.20%, respectively, which is in line with the results of on Podolsk and Belarusian Black-and-White cattle, i.e., 38.3-65 .0% [24], 68.8% [33] and 48.4-80.0% [12].

The average number of cumulus-oocyte complexes per donor heifer was 4.17±0.69 for Simmental cattle and 3.36±0.41 for Yaroslavl cattle, which corresponds to or exceeds the figures established by other authors for Podolsk and Belarusian Black-and-White cattle, 2.67 [33], 3.17 [12], 3.40 [24], 4.80 [12] and 5.00 [2]. The average number of COCs reported by L.N. Rotar et al. [26] for Black-and-White Holsteinized cows was significantly higher and accounted to 11.3.

In our experiment, 85.33% of COCs from Simmental heifers and 81.55% of COCs from Yaroslavl heifers were suitable for embryo production in vitro. These figures in most cases exceeded those established by other researchers. The viable COCs in the Black-and-White Holsteinized cows reached 42.48% [26]. The authors attribute the relatively low quality of COCs to an increased load on the cows due to lactation. The proportion of suitable oocytes reached 79.30% in native Podolsk cattle [24], 67.26% in Aberdeen Angus cows [26], 71.4 and 83.9% the Gir and Nelore cows, respectively [26]. In the report of V.K. Pestis et al. [34], the share of suitable COCs with optimal technical OPU characteristics reached 70.6%. The presence of a relatively larger proportion of usable COCs in our experience may be due to different criteria of the COC quality evaluation. Thus, in most studies, COCs lacking cumulus cells (denuded oocytes) are considered unsuitable for obtaining embryos in vitro [26, 34-36]. In our studies, the main criteria for the quality of COCs were the color and homogeneity of the ooplasm, and therefore denuded oocytes with homogeneous ooplasm were considered conditionally

suitable for IVP procedure.

In our experiment, the number of COCs suitable for embryo production in vitro averaged 3.08±0.60 per donor for the Simmental breed and 2.52±0.29 for the Yaroslavl breed. L.N. Rotar et al. [26] received 11.3 viable COCs per a donor Aberdeen Angus cow and 4.8 COCs per a donor Black Pied Holsteiner cow. In aboriginal Podolsk cattle, an average of 2.70 viable COCs per donor were identified [24], in Aberdeen Angus heifers, an average of 6.8 viable COCs per donor were obtained [37]. In studies on Holsteinized Black-and-White cattle, the number of eligible COCs per donor was 2.03 [33], 2.38 [12], 3.4 [27] and 3.90 [34].

Thus, we revealed a high variability in the results of OPU procedure between donor heifers of both Simmental and Yaroslavl breeds assessed by the average number of ultrasound-visible follicles and the number of oocyte-cumulus complexes obtained. These individual differences between donors were statistically significant. Given the high positive correlation between the number of punctured follicles and extracted retrieved COCs, it is reasonable to select animals with a large number of ultrasound-visible follicles to improve the OPU performance. Comparison of the OPU effectiveness in the donor Simmental and Yaroslavl heifers did not reveal significant quantitative and qualitative differences between the COCs. Therefore, the technical (type of aspiration needle and vacuum pressure) and technological parameters (number of OPU sessions) optimized for Simmental donor heifers can be used for Yaroslavl heifers without any noticeable loss of performance.

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