Does the dominant follicle affect ovum pick-up and *in vitro* embryo production outcomes?

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Abstract

The objective of this study was to evaluate the impact of the presence of dominant follicles on the outcome of *in vitro* embryo production (IVEP) by conducting a comparative analysis of oocyte number, quality, and *in vitro* embryo development in animals with and without dominant follicles in their ovaries during oocyte pick-up (OPU). The study was conducted by evaluating data obtained from OPU applications, and Holstein heifers in good general health were used in the research. The OPU sessions performed were classified according to the presence of dominant follicles in the ovaries. During oocyte retrieval, two groups were formed: group DFA (dominant follicle-absent) without dominant follicles in either ovary, and group DFP (dominant follicle-present) with dominant follicles. The results demonstrate that the group without dominant follicles in the ovaries exhibited a higher number of quality, total, and viable oocytes ($P < 0.05$). In the developmental follow-up conducted after the *in vitro* culture phase, it was determined that the number of cleaved oocytes and blastocysts was higher in the group without dominant follicles in their ovaries ($P < 0.05$). Consequently, it is thought that OPU procedures performed during the phase preceding the establishment of follicular dominance or in ovaries devoid of dominant follicles may positively affect IVEP yield.

Blastocyst yield, follicular atresia, follicular dominance, oocyte quality

Assisted reproductive technologies, particularly oocyte pick-up (OPU) and *in vitro* embryo production (IVEP), are fundamental tools in modern animal husbandry. These technologies are used to accelerate genetic progress, propagate superior productive animals, and preserve valuable genotypes (Stout 2020). Furthermore, these techniques enable the repetitive collection of oocytes from living donors and the production of embryos through *in vitro* fertilisation. Thus, they ensure both the shortening of the generation interval and the sustainability of superior genetic material (Watanabe et al. 2017).

The success of OPU/IVEP depend on the number of oocytes obtained and their biological viability. The developmental capacity of oocytes is closely related to the donor's physiological condition, and the endocrine and morphological structure of the ovary (Motta et al. 2024). The development capacity is influenced by various factors, including the age of the donor, breed, nutritional status, hormonal protocols used, seasonal variability, and frequency of oocyte retrieval (Huang et al. 2023; Sarvari et al. 2024; Çiftçi et al. 2025). However, within these variables, follicular dynamics and the presence of a dominant follicle are particularly important determinants of oocyte cytoplasmic maturation and embryo development potential (Velazquez 2023; Çiftçi et al. 2025).

The dominant follicle suppresses the development of small antral follicles and increases the risk of atresia by altering hormonal balance in the ovary with high oestrogen and inhibin concentrations (Cobo et al. 1999). This phenomenon can lead to a decrease in the number

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and quality of oocytes, and consequently, to a decline in blastocyst development rates (Romero et al. 2018). In contrast, it has been stated that oocytes obtained from ovaries without a dominant follicle or corpus luteum exhibit higher fertilisation rates, cumulus expansion, and embryo quality (Azari-Dolatabad et al. 2023).

The metabolic and biochemical environment, which varies depending on follicular size differences, may form the possible physiological basis for these effects. The amino acid, steroid hormone, and antioxidant profile in follicular fluid changes in accordance with the stage of follicular development. This change is directly related to oocyte metabolism (Mohammed et al. 2019). It has been suggested that the changes in insulin-like growth factor 1 (IGF-1) and progesterone concentrations caused by the dominant follicle may affect the maturation of oocytes in other antral follicles (Azari-Dolatabad et al. 2023). However, how this inhibitory effect is reflected in the developmental competence of the oocyte has not yet been fully defined (Cobo et al. 1999; Romero et al. 2018).

This information indicates the potential for improving efficiency in the evaluation of follicular structure in OPU/IVEP applications. The physiological state and diameter of the follicle are considered fundamental indicators determining oocyte development quality (Motta et al. 2024). The hormone-based suppression exerted by the dominant follicle on other antral follicles is considered to be one of the mechanisms that can limit embryo development (Vennapureddy et al. 2022; Azari-Dolatabad et al. 2023).

The objective of this study was to ascertain the potential impact of dominant follicle presence on IVEP yield. This would be achieved by conducting a comparative analysis of oocyte number, oocyte quality, and *in vitro* embryo development performance in animals with and without dominant follicles in their ovaries during OPU.

Materials and Methods

All procedures performed in this study were approved by the Selçuk University Local Animal Experiment Ethics Committee (approval number 2024/02/33).

Animals and experimental design

This study was conducted by evaluating data obtained from routine OPU procedures. The study included Holstein heifers between 14 and 20 months of age. All animals were maintained in the same herd under similar housing, feeding, and management conditions, and only clinically healthy heifers with no evident reproductive disorder and a body condition score ranging from 2.5 to 3.5 were included. The animals were fed *ad libitum*, and the ration was formulated to include concentrate feed, dry hay, alfalfa silage, corn silage, fresh alfalfa, and vitamin and mineral supplements.

A total of 89 OPU sessions were examined and classified according to the presence of dominant follicles in the ovaries. Each heifer was included only once, and repeated OPU sessions were not performed on the same animal. Because the objective was to compare OPU outcomes according to dominant follicle status, the OPU session was used as the observational unit in the statistical analysis. For the purposes of this study, follicles with a diameter ≥ 10 mm were considered dominant follicles based on an ultrasonographic diameter criterion, consistent with previous descriptions of bovine dominant follicles (McEvoy et al. 2022). Accordingly, 31 sessions (dominant follicle-absent, DFA) in which no dominant follicle was found in either ovary were compared to 58 sessions (dominant follicle-present, DFP) in which a dominant follicle was identified in both ovaries. During each OPU procedure, the ovaries were evaluated ultrasonographically, and sessions were classified according to the presence or absence of a dominant follicle at the time of oocyte retrieval. Follicle diameter measurements were performed using a transvaginal probe. During OPU sessions, follicles with a diameter of at least 2 mm were aspirated for the purpose of oocyte retrieval. The follicular fluid obtained after aspiration was transferred to tubes at 37 °C. The collected oocytes were enumerated under a stereomicroscope and subjected to a quality classification. The total oocyte count, oocyte quality distributions, and *in vitro* embryo development performance were statistically compared between the two groups formed based on the presence of dominant follicles.

Ovum pick-up

In the transvaginal oocyte retrieval procedure, a collection unit integrated into a combined catheter-aspiration system was utilized (Cattle OPU aspiration pump, 230 V, Minitube, Germany). The preferred equipment for ultrasound imaging during follicle diameter measurement and oocyte collection comprised a real-time ultrasound device (Esaote MyLab TwiceVet, Italy) and a microconvex probe (SC3123 VET,

Italy) with a frequency range of 4.0–9.0 MHz. Prior to the OPU procedure, the follicular structures in the ovary were classified according to their diameter, and working groups were formed accordingly. During the collection procedure, all follicles with a diameter ≥ 2 mm in the ovary were aspirated using a 20-gauge catheter needle and a microconvex probe. Oocyte recovery was performed on a random day of the oestrous cycle without any hormonal stimulation.

Classification of oocytes and *in vitro* embryo production

The morphological evaluation of cumulus-oocyte complexes (COCs) was conducted under a stereomicroscope, and oocytes were categorised according to the criteria of cumulus cell compactness, cumulus cell layer structure, and cytoplasmic homogeneity. In this context, oocytes were classified into four quality grades: very good (Grade A), good (Grade B), moderate (Grade C), and degenerate (Grade D) (Petyim et al. 2003). The IVEP procedure exclusively incorporated oocytes classified as Grade A, B, or C. Oocytes that exhibited a homogeneous cytoplasm, regular morphology, and a minimum of one layer of compact cumulus structure were deemed to be viable (Hayden et al. 2022).

Embryo production was performed using the following commercially available media from IVF Bioscience (Cornwall, United Kingdom): BO-OPU, BO-IVM, BO-IVF, BO-IVC, BO-Wash, BO-Oil and BO-SemenPrep. Subsequent to the quality assessment, the selected COCs were washed three times in BO-Wash environment. The oocytes were then placed in BO-IVM medium for *in vitro* maturation and incubated at 38.5 °C and 5.5% CO_2 for 20–22 h. Post-maturation oocytes were transferred to BO-IVF medium for *in vitro* fertilization, and semen from the same bull was prepared in BO-SemenPrep medium and added for fertilization. The fertilization process was conducted at a temperature of 38.5 °C and a CO_2 concentration of 5% for a duration of 20 h.

Subsequent to the process of fertilization, the oocytes were subjected to vortexing in order to remove the cumulus cells. Thereafter, the potential zygotes were transferred to BO-IVC medium for *in vitro* culture. Potential zygotes were incubated in a culture system covered with BO-Oil at 38.5 °C, 6% CO_2, and 6% O_2 throughout the *in vitro* culture period. The cleavage rate was evaluated on day 7 of *in vitro* culture, while blastocyst formation was assessed on days 7 and 8 of culture. The stage of development and embryo quality were assessed according to the criteria of the International Embryo Technology Society (Bó and Mapletoft 2013; Alkan et al. 2025).

Statistical analysis

Statistical analysis of the data was conducted using the SPSS 27.0 program (IBM SPSS Statistics for Windows, Version 27.0., IBM Corp., Armonk, NY, USA). The data obtained in the study were evaluated in terms of basic assumptions prior to analysis. Descriptive statistics are expressed as means ± standard error of the mean (SEM). Independent samples *t*-test was employed to evaluate the differences between groups. For the tests, a *P* value of less than 0.05 was considered to be significant.

Results

The average oocyte numbers and quality distributions obtained from donor animals without a dominant follicle in the ovary and with a dominant follicle during oocyte collection sessions are presented in Table 1. In the course of the evaluation, it was established that the mean total number of oocytes obtained per OPU session was higher in the DFA group than in the DFP group ($P < 0.05$). Statistical evaluations revealed that the number of oocytes in Grade A and Grade B quality classes obtained per OPU session was significantly higher in the DFA group compared to the DFP group. In contrast, no significant difference was found between the two groups in terms of the average number of Grade C and Grade D oocytes. The percentage distribution of oocytes in both groups based on morphological properties is shown in Fig. 1. In addition, the average number of viable oocytes considered suitable for use in *in vitro* embryo production was also found to be higher in the DFA group.

The data obtained following *in vitro* embryo production processes are presented in Table 2. In the subsequent development assessment conducted after the *in vitro* culture phase, it was determined that the average number of cleaved oocytes obtained from oocytes belonging to the DFA group was higher than that of the DFP group ($P < 0.05$). When the average number of blastocysts evaluated in the advanced stages of embryo development was examined, a similar trend was observed, and the number of blastocysts obtained per session in the DFA group was found to be higher than in the DFP group ($P < 0.05$).

Table 1. Numbers of morphologically classified oocytes per OPU session in DFA and DFP groups (data are presented as means ± SEM).

	Grade A	Grade B	Grade C	Grade D
Group DFA	4.90 ± 0.94	3.61 ± 0.53	3.48 ± 0.66	3.87 ± 0.53
Group DFP	2.44 ± 0.32	2.24 ± 0.34	3.12 ± 0.35	3.62 ± 0.42
P value*	0.004	0.027	0.59	0.70

OPU - ovum pick-up; DFA - dominant follicle-absent; DFP - dominant follicle-present; SEM - standard error of the mean

*Significance was set at $P < 0.05$

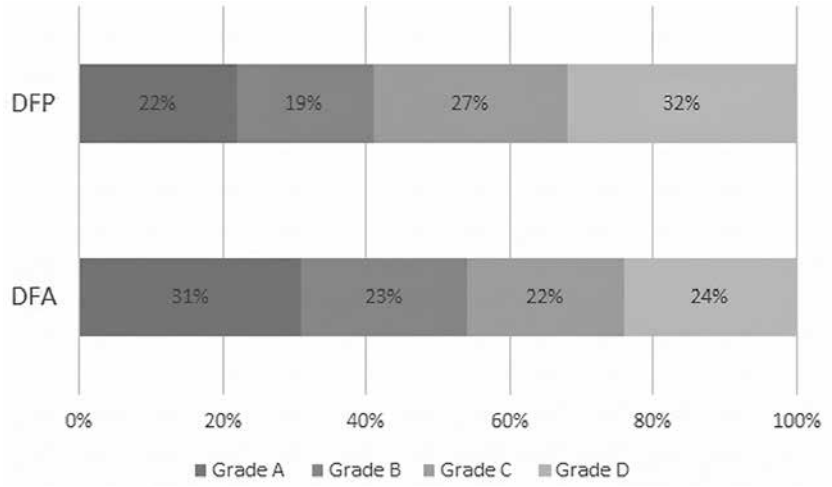

Fig. 1. Percentage distribution of oocytes classified according to their morphological characteristics within groups

Table 2. OPU/IVEP outcomes in DFA and DFP groups (data are presented as means ± SEM).

	Group DFA	Group DFP	*P* value*
Total oocytes / OPU	15.87 ± 1.74	11.43 ± 1.09	0.027
Viable oocytes / OPU	11.51 ± 1.47	7.84 ± 0.78	0.017
Cleaved oocytes / OPU	8.32 ± 0.89	5.41 ± 0.61	0.008
Blastocysts / OPU	2.71 ± 0.41	1.32 ± 0.25	0.003

OPU - ovum pick-up; IVEP - *in vitro* embryo production; DFA - dominant follicle-absent; DFP - dominant follicle-present; SEM - standard error of the mean

*Significance was set at $P < 0.05$

Discussion

The present study established that the number of A and B quality oocytes per OPU, the total and viable oocyte count, the number of cleaved oocytes, and the number of blastocysts were higher in donors without dominant follicles in their ovaries compared to the group with dominant follicles. The findings suggest that the suppressive effect of the dominant follicle on other antral follicles extends beyond follicular dynamics to also influence oocyte

competence and *in vitro* embryo development performance. These results are generally consistent with studies in the literature reporting that the dominant follicle has adverse effects on oocyte and embryo development in bovine ovaries (Aguila et al. 2020).

The higher total oocyte count in the group without dominant follicles can be attributed to the selection dynamics of the follicular wave. The dominant follicle continues to develop, selected from among the FSH-dependent growing follicles, while the remaining small follicles experience suppressed growth and accelerated atresia (Beg and Ginther 2006). Research has indicated that the number of oocytes obtained may increase when follicular wave development is inhibited during the early growth phase or when dominant follicle development is suppressed (Fernandes et al. 2020). Saleem et al. (2022) reported that proper timing of the follicular wave before OPU and reducing the suppressive effect of the dominant follicle can increase oocyte number and IVF success rates. Additionally, in studies using FSH superstimulation or wave synchronization, delaying or eliminating the dominant follicle has been shown to increase the number of follicles and oocytes per OPU (Krishna et al. 2023).

Beyond the increase in total oocyte number, the higher number of A and B quality oocytes in the group without a dominant follicle may be attributed to the ability of small- and medium-sized follicles to develop in a more homogeneous hormonal and paracrine environment. In the presence of a dominant follicle, increased oestrogen and inhibin secretion suppress FSH levels, leading to increased atresia in subordinate follicles in the same wave and decreased oocyte competence (Kanitz 2003; Garcia-Guerra et al. 2018). Hagemann (1999) reported that the proportion of apoptotic cells in subordinate follicles increased during the dominant phase and that the blastocyst development rates of oocytes obtained in this phase were significantly lower than those collected during the follicular growth phase. In a similar way, Hendriksen et al. (2004) stated that the *in vitro* development capacity of oocytes obtained during the late dominant phase of the follicular wave was diminished. Recent reviews focusing on oocyte selection have emphasized that the presence of a dominant follicle can negatively affect the embryo development performance of oocytes obtained from other follicles in the ovary (Aguila et al. 2020). In this context, the presence of a higher number of A and B quality oocytes from ovaries without a dominant follicle suggests that the follicular population matures in a more synchronized and less stressful microenvironment, which appears consistent with the literature.

The higher number of viable oocytes in the group without dominant follicles indicates that the increase is not only numerical, but also affects the oocyte capacity suitable for *in vitro* embryo production in the follicular microenvironment. The metabolic profile of follicular fluid (energy metabolites, amino acids, lipids and antioxidants) is closely related to oocyte cytoplasmic maturation and cell membrane integrity (Read et al. 2021). Recent metabolomics studies have demonstrated that in conditions of negative energy balance, oxidative stress, or hormonal imbalance, there is disruption to the composition of follicular fluid, which in turn reduces oocyte viability and developmental competence (Marei et al. 2022; Lu et al. 2025). Increased apoptosis and impaired steroidogenesis in subordinate follicles in the presence of a dominant follicle are proposed as possible mechanisms explaining the decrease in the proportion of viable oocytes (Hagemann 1999). Although hormonal or metabolic indices were not directly measured in our study, the higher yield of viable oocytes from ovaries without a dominant follicle is consistent with this physiological background.

The higher number of cleaved oocytes in the group without a dominant follicle suggests that the nuclear and cytoplasmic maturation level of the oocyte may be associated with its early embryonic division capacity following *in vitro* fertilization. The cleavage rate is considered a practical indicator of oocyte competence. It has also been reported that division proceeds more synchronously and rapidly in zygotes derived from high-quality

oocytes (Pytel et al. 2024). The initial developmental stage is influenced by factors such as follicle size, wave phase, and the presence of a dominant follicle. These factors operate through the oocyte's mitochondrial function and mRNA/protein reserves (Ghanem et al. 2007). Hagemann (1999) and Hendriksen et al. (2004) have stated that cleavage and further development rates in embryos obtained from oocytes collected during the growth phase may be higher than those collected during the dominant phase.

The higher number of blastocysts observed in the group without dominant follicles suggests that the benefits in oocyte quality and early embryonic development can be sustained until the blastocyst stage. The development of the blastocyst is dependent on the coordinated progression of mitochondrial activity, energy metabolism, genome activation, and the differentiation of the trophoectoderm and inner cell mass (Ghanem et al. 2007; Lu et al. 2025). Research in both classical and contemporary studies supports the notion that the quality of the follicular environment, particularly the oestradiol/progesterone balance and local growth factors, is associated with blastocyst development rates (Del Collado et al. 2018; Latorraca et al. 2025). Hendriksen et al. (2004) reported that blastocyst rates were significantly lower in oocytes obtained during the dominant phase, whereas higher development rates were achieved during the growth phase or in the absence of dominant follicle suppression. Furthermore, Aguila et al. (2020) emphasize that the presence of a dominant follicle may also have a detrimental effect on the blastocyst development potential of oocytes collected from other follicles in the same ovary. A similar study on patients with polycystic ovary syndrome found that ovarian follicular dominance reduced the usable blastocyst rate (Romero et al. 2018).

As a result, in the absence of a dominant follicle, the counts of Grade A-quality, Grade B-quality, and viable oocytes, as well as total oocytes, cleaved oocytes, and blastocysts, were found to be higher. The findings suggest that OPU procedures performed during the phase preceding follicular wave dominance or in ovaries stripped of dominant follicles may positively affect IVEP efficiency.

However, the focus of this study has been on comparing oocyte and embryo development data in practice, rather than at the hormonal and molecular levels. Therefore, mechanistic interpretations regarding the effect of the dominant follicle are based on the physiological background reported in the literature. Nevertheless, the results obtained may provide a clinically meaningful basis for future studies, including hormonal profiling, follicular fluid composition, and gene expression analysis.

Conflict of interest

None of the authors have any conflict of interest to declare.

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