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ORIGINAL ARTICLE

Optimization of ultrasound guided ovum pick up technique in Bangladeshi crossbred dairy cattle

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Abstract

Background: Ovum pick-up (OPU) is a key assisted reproductive technology (ART) for accelerating genetic improvement in dairy cattle, though its efficiency depends on follicular dynamics and oocyte quality. The study highlighted that OPU technology remains underutilized in Bangladesh, where oocytes for embryo production have traditionally been collected from slaughterhouse-derived ovaries. However, ultrasound-guided OPU allows controlled and repeated oocyte recovery from live donor animals of known genetic merit. In tropical systems like Bangladesh, variability may constrain outcomes. This study evaluated estrogen-based follicular wave synchronization on follicular characteristics, oocyte recovery, and oocyte quality in Bangladeshi crossbred dairy cattle.

Methods: Eighteen clinically healthy Bangladeshi crossbred dairy cows were subjected to two OPU sessions at 14 days intervals: (1) control (non-stimulated) and (2) hormone stimulated following insertion of a progesterone releasing intravaginal device (CIDR®) combined with estradiol benzoate and cloprostenol administration. Follicles were counted at Day 5 post-synchronization using transrectal ultrasonography. Recovered cumulus-oocyte complexes (COCs) were morphologically graded according to IETS standards. The data were analyzed using an independent samples t-test to compare the means between the control and hormone-stimulated groups.

Results: Hormone-synchronized sessions produced higher mean follicle numbers and oocyte recovery than control sessions in both animal categories. Mean total follicle number increased from 24.10 to 37.40 in heifers and from 28.70 to 42.10 in cows. Mean recovered oocytes increased from 10.20 to 18.30 in heifers and from 12.50 to 21.10 in cows. Recovery rate also increased from 42.32% to 48.93% in heifers and from 43.55% to 50.12% in cows.

Conclusion: Estrogen-based follicular wave synchronization prior to OPU significantly improves follicular coordination, increases oocyte recovery efficiency, and enhances the yield of high quality oocytes in Bangladeshi crossbred dairy cattle. Implementation of estrogen synchronization protocols may optimize OPU-IVP systems and support sustainable genetic improvement under tropical production conditions.

Keywords: Assisted reproductive technology; cattle; estradiol; oocyte recovery; ovum pick-up; synchronization.

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Introduction

Assisted reproductive technologies (ARTs) have become essential tools for accelerating genetic improvement and enhancing reproductive efficiency in modern dairy cattle production systems. These technologies, including ovum pick-up (OPU), in vitro embryo production (IVP), and embryo transfer (ET), enable rapid multiplication of genetically superior animals, shorten generation intervals, and increase selection intensity within breeding programs (Ciornei, 2021; Sharma et al., 2024; Salek et al., 2025). In recent years, IVP has become the dominant method of embryo production globally due to its efficiency, flexibility, and ability to produce embryos from a wide range of donor animals, including prepubertal, pregnant, and lactating females (Motta et al., 2025). These technological advancements have significantly improved the dissemination of desirable genetic traits and contributed to sustainable livestock production.

Among ART techniques, OPU combined with IVP has emerged as a powerful approach for maximizing the reproductive output of genetically superior donor animals. OPU allows transvaginal ultrasound-guided aspiration of ovarian follicles in live animals, enabling repeated recovery of oocytes without requiring estrus detection or extensive superovulatory treatments (Kasimanickam and Boler, 2024). This technology allows more efficient utilization of elite donor animals and supports continuous embryo production throughout the reproductive cycle. The success of OPU-IVP systems largely depends on the number and developmental competence of oocytes recovered, which are strongly influenced by ovarian follicular population dynamics and physiological status of the donor (Hayden, 2022; Salek et al., 2025). Prior to the introduction of OPU technology in Bangladesh, oocytes for in vitro embryo production were primarily collected from ovaries obtained at slaughterhouses. This method provided a practical and economical source of oocytes for laboratory-based embryo production studies, particularly during the early development of reproductive biotechnology in the country. Research conducted using slaughterhouse-derived ovaries demonstrated successful in vitro maturation and fertilization of oocytes from indigenous zebu and crossbred cattle, confirming the feasibility of applying ART under local conditions (Das et al., 2006; Morshed et al., 2014). However, slaughterhouse-derived oocytes have several limitations, including unknown donor identity, variable reproductive and physiological status, and inability to repeatedly utilize genetically superior animals. These constraints limit their effectiveness for systematic genetic improvement programs. In contrast, OPU enables controlled and repeated oocyte recovery from live donor animals of known genetic merit, making it a more effective tool for targeted embryo production and genetic advancement (Kasimanickam and Boler, 2024).

The efficiency of OPU is closely associated with ovarian follicular dynamics, which are regulated by complex endocrine interactions involving follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, and progesterone (Wiltbank et al., 2014; Sharma et al., 2026). Follicular development occurs in a wave-like pattern characterized by recruitment, selection, and dominance of follicles. During each wave, a dominant follicle suppresses the growth of subordinate follicles, thereby reducing the number of follicles available for aspiration and contributing to variability in oocyte recovery (Bó and Mapletoft, 2019). This physiological dominance results in asynchronous follicular development, which negatively affects the efficiency and consistency of oocyte recovery and embryo production (Hayden, 2022; Salek et al., 2025). To overcome these limitations, hormonal synchronization protocols have been developed to regulate follicular wave emergence and improve oocyte recovery efficiency. Estradiol-based synchronization protocols, often combined with progesterone supplementation, induce regression of dominant follicles and promote synchronized emergence of a new follicular wave (Bó et al., 2019; Patterson and Thomas, 2019). Several studies have demonstrated that synchronization of follicular wave emergence prior to OPU improves oocyte recovery rates, enhances oocyte developmental competence, and increases embryo production efficiency (Garcia et al., 2025; Hayden, 2022; Motta et al., 2025). Oocyte quality is a critical determinant of successful embryo production. Developmental competence of oocytes is influenced by follicle size, follicular microenvironment, and endocrine status. Oocytes derived from medium-to-large follicles generally exhibit higher developmental potential, improved fertilization rates, and increased embryo production compared with those obtained from smaller follicles (Motta et al., 2025; Salek et al., 2025). Synchronization of follicular wave emergence promotes coordinated follicular growth and enhances oocyte developmental competence, thereby improving overall efficiency of OPU-IVP systems (Vieira et al., 2014; Hayden, 2022).

Despite significant global advancements in reproductive biotechnology, the application of OPU-IVP technology in developing countries such as Bangladesh remains limited. The dairy cattle population of Bangladesh consists predominantly of crossbred animals, particularly Holstein-Friesian crosses, which exhibit improved milk production potential but often demonstrate variable reproductive performance under tropical environmental conditions (Juyena et al., 2018; Maoya et al., 2020). Environmental stress, nutritional imbalance, and management limitations can adversely affect ovarian function, follicular development, and oocyte quality, thereby reducing reproductive efficiency (Salek et al., 2025). Furthermore, most studies on follicular synchronization and OPU-IVP have been conducted in temperate regions under intensive management systems, and limited information is available regarding their effectiveness in crossbred cattle managed under tropical conditions.

Therefore, the present study was designed to evaluate the effects of estrogen-based follicular wave synchronization prior to ovum pick-up on follicular population characteristics, oocyte recovery efficiency, and oocyte quality recovery rate in Bangladeshi crossbred dairy cattle. The findings of this study are expected to contribute to the development of optimized, field-applicable OPU-IVP protocols and support the wider adoption of advanced reproductive technologies for genetic improvement and sustainable dairy production in Bangladesh.

Materials and Methods

Ethical approval

The research design was based on guidance provided by the UK Animals (Scientific Procedures) Act, 1986, and the EU Directive 2010/63/EU, and approved by the Animal Welfare and Experimentation Ethics Committee Bangladesh Agricultural University, Ref. no. AWEEC/BAU/2022(03).

Study area and period

The study was carried out at research animal farm of the department of Surgery and Obstetrics, Bangladesh Agricultural University Mymensingh-2202, and commercial dairy farms at Kishorgonj and Rangpur district from July 2024 to June 2025.

Experimental animals and selection criteria

A total of 18 (8 heifers and 10 cows) clinically healthy Bangladeshi Holstein-Friesian crossbred cattle were used as oocyte donors in this study. Animals were selected based on predefined criteria, including non-pregnant status, regular estrous cyclicity confirmed by transrectal ultrasonography, absence of reproductive or ovarian abnormalities, an acceptable physiological body condition score, and no history of hormonal treatment for at least one estrous cycle prior to the experiment. All animals were maintained under similar feeding, housing, and management conditions throughout the experimental period to minimize environmental variation.

Experimental design

Each donor animal underwent two ovum pick-up (OPU) sessions at 14 days intervals under distinct ovarian conditions: (1) a control session performed during a spontaneous follicular wave without hormonal intervention, and (2) a hormone-stimulated session conducted following estrogen treatment to synchronize follicular wave emergence. The same animals were used in both sessions, enabling within-animal comparisons of follicular dynamics and oocyte recovery parameters.

Estradiol-based follicular wave synchronization

For estrogen-stimulated ovum pick-up (OPU) sessions, on Day 0, a controlled internal drug release device CIDR® (Zoetis, Brazil); contains 1.38 g progesterone was inserted intravaginally. Concurrently, Cidirol® (Zoetis, Brazil), an injectable estradiol benzoate preparation containing 1 mg/ml, was administered at a dose of 2 mg per animal (2 ml) and prostaglandin (Estrumate®) 150 µg cloprostenol sodium was injected intramuscularly. This estradiol benzoate-based synchronization protocol is widely used in cattle for controlling follicular dynamics before OPU and embryo transfer (ET) procedures and has been shown to increase the proportion of aspiratable follicles and improve oocyte recovery efficiency. On Day 5, Cumulus-Oocyte Complexes (COCs) collection was carried out using a standard transvaginal ultrasound-guided follicular aspiration technique. Animals were appropriately restrained to ensure safety and minimize stress. A transvaginal ultrasound probe (6.5 MHz) Image fitted with a needle (18 gauge) guide was used to visualize ovarian follicles, and all follicles with a visible antrum were aspirated using a sterile needle connected to a vacuum pump. Aspiration pressure was standardized across all sessions to maintain consistency. During each OPU session, all visible follicles on both ovaries were aspirated.

Follicular assessment and classification

Immediately prior to OPU, follicles were counted and classified according to International Embryo Technology Society (IETS) Manual 2022. The number of follicles were recorded separately for the non-stimulated and estrogen-stimulated sessions.

Oocyte recovery and classification

Recovered follicular fluid was examined under a stereomicroscope (Olympus SZX2-TR30, Tokyo, Japan) to identify and collect cumulus-oocyte complexes (COCs), and the total number of COCs recovered per session was recorded. COCs were then evaluated for developmental stage and morphological quality in accordance with the International Embryo Technology Society (IETS) Manual (2022) and classified as follows: Grade A (compact, multilayered cumulus cells with homogeneous ooplasm), Grade B (partial cumulus investment with minor irregularities), Grade C (sparse or incomplete cumulus layers), and degenerated (denuded or morphologically abnormal oocytes). COC grading was performed immediately after recovery to minimize handling-related effects.

Oocyte recovery rate

Oocyte recovery rate (%) was calculated for each OPU session using the following formula:

$$\text{Recovery rate (\%)} = (\text{number of oocytes recovered / total number of follicles aspirated}) \times 100.$$

Statistical analysis

The data were analyzed using an One-way ANOVA (4 groups). Results are presented as mean ± standard deviation (SD). Differences were considered statistically significant at $P < 0.05$. Means with different superscripts (a, b, c) differ significantly within the same row indicate significant differences between groups.

Table 1: Summary of the experimental design.

Animal category	Number of donors	Control session	Synchronized session	Interval
Heifers	8	OPU during spontaneous follicular wave	CIDR + estradiol benzoate + prostaglandin; OPU on Day 5	14 days
Cows	10	OPU during spontaneous follicular wave	CIDR + estradiol benzoate + prostaglandin; OPU on Day 5	14 days

Results

Animal category and hormonal stimulation significantly influenced OPU performance in crossbred dairy cattle (Table 2). The highest mean total follicles were observed in the cow hormone-stimulated group (42.10 ± 1.20), followed by the heifer hormone-stimulated group (37.40 ± 1.02). Lower values were recorded in cow control (28.70 ± 0.95) and heifer control groups (24.10 ± 0.88).

Similarly, the number of oocytes recovered was significantly greater in hormone-treated groups. Cow hormone-stimulated animals yielded the highest number of oocytes (21.10 ± 0.70), followed by heifer hormone-stimulated animals (18.30 ± 0.61). Control groups showed significantly lower recovery (12.50 ± 0.52 and 10.20 ± 0.47 for cow and heifer controls, respectively).

Recovery rate was also significantly improved by hormonal stimulation. The highest recovery rates were observed in cow hormone-stimulated (50.12 ± 0.96%) and heifer hormone-stimulated groups (48.93 ± 1.05%), while control groups recorded lower rates (43.55 ± 1.18% and 42.32 ± 1.25%). One-way ANOVA indicated highly significant differences among groups for all parameters ($p < 0.001$).

Discussions

The present findings clearly demonstrate that hormonal stimulation significantly enhances OPU performance in crossbred dairy cattle. Increased follicle numbers in stimulated groups indicate improved follicular recruitment due to exogenous gonadotropin treatment, which promotes synchronized growth of multiple follicles. OPU with 14-day intersession intervals increased the quality of embryos after in cows, especially on the follicle number and COC morphology (Anchordoquy et al., 2024). These findings confirm that endocrine manipulation of follicular wave emergence improves both the efficiency and developmental competence of recovered oocytes. Synchronization of follicular waves is a critical factor influencing OPU success because the number, size, and physiological condition of follicles at the time of aspiration directly affect oocyte yield and quality (Bó and Mapletoft, 2019; Salek et al., 2025). The higher oocyte recovery observed in cows compared with heifers may be associated with greater ovarian maturity, larger ovarian reserve, and improved follicular responsiveness in mature animals. This suggests that cows are more suitable donors for intensive embryo production programs.

Effect of estrogen stimulation on follicular population characteristics

One of the most significant effects observed in the present study was the increase in total follicle number following estrogen stimulation compared with non-stimulated sessions. Similar observations have been reported in previous stud-

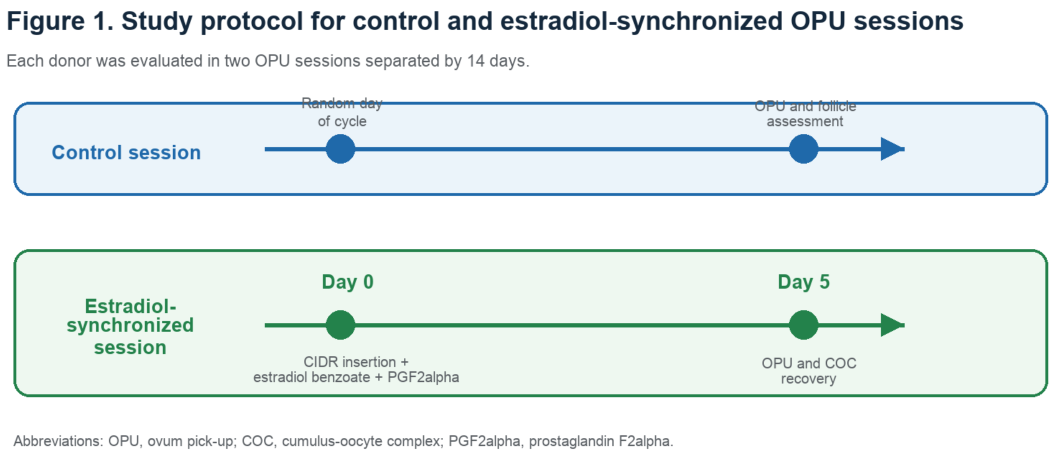

Figure 1: Study protocol for control and estradiol-synchronized OPU sessions.

Table 2: Effects of hormonal stimulation on OPU performance in crossbred dairy cattle.

Group	Total follicles	Oocytes recovered	Recovery rate (%)
Heifer Control	24.10 ± 0.88^{c}	10.20 ± 0.47^{c}	42.32 ± 1.25^{c}
Heifer Hormone-Stimulated	37.40 ± 1.02^{b}	18.30 ± 0.61^{b}	48.93 ± 1.05^{a}
Cow Control	28.70 ± 0.95^{c}	12.50 ± 0.52^{c}	43.55 ± 1.18^{c}
Cow Hormone-Stimulated	42.10 ± 1.20^{a}	21.10 ± 0.70^{a}	50.12 ± 0.96^{a}

Data expressed as Mean +/- SE. Means with different superscripts (a, b, c) differ significantly.

ies. Garcia et al. (2025) demonstrated that synchronization of follicular wave emergence prior to OPU increased the number of follicles suitable for aspiration and improved oocyte competence. Motta et al. (2025) further emphasized that synchronization enhances follicular coordination and improves ovarian responsiveness in OPU-IVP systems. Larger follicles provide a more favorable microenvironment for oocyte maturation, including enhanced granulosa cell activity and endocrine support (Salek et al., 2025). Therefore, the increased number of large follicles observed in this study indicates improved follicular synchronization and enhanced ovarian function.

Effect of estrogen stimulation on oocyte recovery and recovery efficiency

Consistent with the observed changes in follicular population dynamics, hormone stimulated sessions resulted in significantly higher oocyte recovery and improved recovery efficiency compared with control sessions (Table 2). Oocyte recovery efficiency depends on follicular population characteristics and synchronization status at the time of OPU (Hayden, 2022; Salek et al., 2025). Guest et al. (2024) reported that synchronization and repeated OPU procedures improved follicular coordination and maintained stable oocyte recovery rates. Similarly, synchronization protocols increase the proportion of follicles reaching optimal developmental stages, thereby improving oocyte quality and embryo production potential (Garcia et

Table 3: Descriptive change from control to synchronized OPU sessions.

Animal category	Change in follicles	Change in recovered oocytes	Change in recovery rate	Interpretation
Heifers	+13.30 follicles	+8.10 oocytes	+6.61 % points	Synchronization improved all recorded OPU indicators.
Cows	+13.40 follicles	+8.60 oocytes	+6.57 % points	Cows showed the highest numerical post-synchronization values.

al., 2025).

Implications for OPU programs in Bangladeshi crossbred dairy cattle

The experiment demonstrates that hormonal stimulation prior to OPU significantly improves both the quantity and quality of recovered oocytes in Bangladeshi crossbred dairy cattle (Table 3). Crossbred cattle often exhibit variable ovarian responses due to genetic and environmental factors. Synchronization protocols improve follicular coordination and enhance reproductive efficiency under field conditions. These improvements are particularly important in tropical production systems, where reproductive performance may be influenced by environmental and physiological stressors.

Although estrogen stimulation improved follicular synchronization and oocyte recovery and recovery rate assessment in this study was based on morphological grading. Future studies should evaluate embryo development outcomes, including fertilization, cleavage, blastocyst formation, and pregnancy success, to confirm the functional benefits of estrogen synchronization. Additionally, donor-related factors such as metabolic status, ovarian reserve, and physiological condition should be evaluated to improve reproductive efficiency and optimize OPU outcomes. These results are highly relevant for Bangladesh, where advanced reproductive biotechnologies remain underutilized. Strategic use of hormone-stimulated donor cows could maximize OPU efficiency and support rapid genetic improvement of dairy cattle population (Salek et al., 2025; Motta et al., 2025).

Conclusion

The present study demonstrated that hormonal stimulation prior to ovum pick-up significantly improved total follicle numbers, oocyte recovery, and recovery rate in Bangladeshi Holstein-Friesian crossbred cattle. Among all groups, hormone-stimulated cows showed the best overall performance, indicating their superior suitability as donor animals for embryo production programs. Therefore, the use of hormonally stimulated mature cows is recommended to optimize OPU efficiency and accelerate genetic advancement in Bangladeshi dairy cattle.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. Anchordoquy JP, Balbi M, Farnetano NA, Giuliodori MJ, Nikoloff N, Carranza-Martín AC, Furnus CC, Anchordoquy JM, 2024. Effect of follicular wave synchronization using a progesterone-releasing intravaginal device-based protocol on in vitro embryo production in *Bos taurus* cows subjected to 14-day interval ovum pick-up. *Animal Reproduction* 21(1): e20240012. https://doi.org/10.1590/1984-3143-AR2024-0012
2. Bó GA, Baruselli PS, Mapletoft RJ, 2019. Superovulation and embryo transfer in cattle: Current perspectives. *Animal Reproduction* 16(3): 423–440. https://doi.org/10.21451/1984-3143-AR2019-0050
3. Bó GA, Mapletoft RJ, 2019. Historical perspectives and recent research on superovulation in cattle. *Theriogenology* 133: 48–61. https://doi.org/10.1016/j.theriogenology.2019.04.020
4. Ciornei SG, 2021. Advances in reproductive biotechnology and embryo production in cattle. *Reproduction in Domestic Animals* 56(Suppl 3): 15–24. https://doi.org/10.1111/rda.13945
5. Das ZC, Siddiqui MA, Bhattacharjee J, Bhuiyan MMU, Shamsuddin M, 2006. In vitro fertilization using zebu and crossbred bull semen. Bangladesh Society for Veterinary Education and Research, Publication 31: 31.
6. Garcia SM, Motta JCL, Silva LHM, Baruselli PS, Mapletoft RJ, Bó GA, 2025. Follicular wave synchronization improves oocyte competence and embryo production in cattle. *Animal Reproduction* 22(1): e20250020. https://doi.org/10.1590/1984-3143-AR2025-0020
7. Guest A, Salek F, Johnson C, Kastelic JP, Thundathil J, 2024. Repeated superstimulation facilitated ovum pick-up and in vitro production of viable bovine embryos with no apparent adverse effects on donor health. *Preprints*. https://doi.org/10.20944/preprints202401.0308.v1
8. Hayden SS, 2022. Influence of follicular population dynamics on oocyte competence and embryo production in cattle. *Theriogenology* 189: 90–101. https://doi.org/10.1016/j.theriogenology.2022.05.018
9. Juyena NS, Ahmed JU, Sutradhar BC, Talukder S, Juyena NS, 2018. Recent advances in assisted reproductive technologies in cattle. *Bangladesh Veterinary Journal* 52(1–4): 1–9.
10. Kasimanickam R, Boler DD, 2024. Ovum pick-up and in vitro embryo production in cattle: Current technologies and applications. *Veterinary Clinics of North America: Food Animal Practice* 40(1): 1–16. https://doi.org/10.1016/j.cvfa.2023.11.002
11. Maoya W, Mebratu B, Desta A, 2020. Application of assisted reproductive technologies in tropical cattle production systems. *Tropical Animal Health and Production* 52(4): 1805–1815. https://doi.org/10.1007/s11250-020-02245-7
12. Morshed SN, Bhuiyan MMU, Rahman MM, Singha JK, Juyena NS, 2014. Culture conditions for in vitro maturation of abattoir-derived oocytes of native zebu cows of Bangladesh. *Journal of Embryo Transfer* 29(3): 201–206. https://doi.org/10.12750/JET.2014.29.3.201
13. Motta JCL, Cosentino IO, Martins JP, Vieira LM, Sá Filho MF, Baruselli PS, Mapletoft RJ, Bó GA, 2025. Hormonal synchronization and its effects on follicular development and oocyte competence in cattle. *Animal Reproduction* 22(2): e20250045. https://doi.org/10.1590/1984-3143-AR2025-0045
14. Patterson DJ, Thomas JM, 2019. Control of estrous cycle and follicular wave synchronization in cattle. *Veterinary Clinics of North America: Food Animal Practice* 35(1): 1–17. https://doi.org/10.1016/j.cvfa.2018.10.002
15. Salek F, Johnson C, Kastelic JP, Thundathil J, 2025. Physiological and management factors affecting ovum pick-up and in vitro embryo production in cattle. *Animal Reproduction Science* 254: 107269. https://doi.org/10.1016/j.anireprosci.2024.107269
16. Sharma R, Kumar B, Biswas N, Reddy AL, Yadav BK, Bajia NP, Vandana, Khan MH, 2024. Assisted reproductive techniques and their application in large ruminants. *International Journal of Veterinary Sciences and Animal Husbandry* 9(4): 410–415.
17. Vieira LM, Rodrigues CA, Castro Netto A, Guerreiro BM, Silveira CRA, Moreira RJ, Sá Filho MF, Bó GA, Mapletoft RJ, Baruselli PS, 2014. Superstimulation prior to ovum pick-up to improve in vitro embryo production in Holstein cows. *Theriogenology* 82(4): 654–660. https://doi.org/10.1016/j.theriogenology.2014.05.012
18. Wiltbank MC, Souza AH, Carvalho PD, Cunha AP, Giordano JO, Fricke PM, Baez GM, Diskin MG, 2014. Physiological and practical effects of progesterone on reproduction in dairy cattle. *Animal* 8(Suppl 1): 70–81. https://doi.org/10.1017/S1751731114000585