

Comparative Assessment of Follicular Activity, Oocyte Recovery and Embryo Production in Friesian Heifers and Adult Cows Following Different OPU Intervals

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Abstract

Study was conducted to evaluate the influence of donor age and Ovum pick-up (OPU) session interval on follicular dynamics, oocyte recovery and in vitro embryo production in Friesian cattle. A total of ten clinically healthy Friesian females were included in the study and categorized into two age groups: heifers aged 12–25 months and adult cows aged 8–10 years. OPU was performed at two different intervals, i.e., 14 days (Group A) and 21 days (Group B) to determine the effects of collection frequency on ovarian response and subsequent embryo production. Selected oocytes were subjected to in vitro maturation, fertilization, and embryo culture procedures. The number of follicles aspirated, oocytes recovered, oocyte recovery rate, cleavage rate, and blastocyst production were recorded and compared according to donor age and OPU interval. The study was executed during the winter season from November to February to minimize the potential confounding effects of environmental heat stress on follicular development and embryo production. The findings of the study are expected to provide useful information regarding the selection of appropriate donor age and OPU frequency for optimizing oocyte yield and embryo production in Friesian cattle. The results may contribute to the development of efficient OPU-based IVEP protocols for enhanced reproductive performance and genetic dissemination in dairy cattle.

Keywords: Catalytic Nanocage Reactor, Hopf Bifurcation, Nonlinear Dynamics, Optimal Control, Machine Learning-Assisted Optimization

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Introduction

OPU followed by in-vitro embryo production (IVEP) is an important reproductive biotechnology used to improve the genetic potential of dairy cattle which is considered more safe as compared to MOET (multiple ovulation and embryo transfer) due to numerous factors. IVEP from oocytes obtained by transvaginal ultrasound-guided retrieval (OPU) is an alternative to the MOET technique basing on the embryo flush from donor cows after multiple ovulation and fertilization within donor genitalia through artificial insemination (AI). OPU carried out through vacuum aspiration with a needle inserted into the bovine follicle. The major advantage of the IVEP compared to the MOET technology is a larger number of embryos during a certain period of time even without using hormones for super-stimulation. The advantage of without changing the normal physiology of cow has become one of the main reasons for rapid replacement of MOET to IVEP. The efficiency of OPU programs is influenced by several factors, including the age of the donor animal, the interval between successive OPU sessions, ovarian follicular dynamics, and the quality of recovered oocytes. Younger animals generally possess a larger ovarian reserve and a higher number of recruit-able follicles, which may lead to better embryo production outcomes than older animals. The ability to obtain oocytes with developmental competence from calves has been recognized for more than 60 years (Baruselli et al 2021). The effectiveness of IVEP technology largely depends on the number and quality of ova harvested from one donor per sessions (Chinarov, 2024).

The present study compared follicular yield and embryo production in Friesian heifers (12–25 months) and adult cows (8–10 years). Ten animals were maintained under identical management, feeding, and environmental conditions. Oocyte collection was performed at two different intervals, i.e 14 days (Group A) and 21 days (Group B), to evaluate the effects of donor age and collection frequency on ovarian response and embryo production.

Material and Method

OPU was performed as a non-surgical, ultrasound-guided technique for the transvaginal recovery of cumulus–oocyte complexes from live Friesian donor

cows. Prior to the procedure, animals were appropriately restrained, the rectum was evacuated, and the perineal region was cleaned using standard hygienic procedures. Epidural anesthesia was administered to minimize discomfort and facilitate ovarian manipulation. A lubricated transvaginal ultrasound probe equipped with a needle guide was introduced into the vagina, while each ovary was manually positioned against the vaginal wall through rectal manipulation to facilitate visualization of the ovarian follicles. Follicles suitable for aspiration were identified ultrasonographically and punctured using a sterile aspiration needle. Follicular fluid containing oocytes was aspirated under controlled vacuum into a pre-warmed collection system containing appropriate collection medium. The aspiration line was periodically flushed with collection medium to maintain efficient recovery. The recovered follicular fluid was maintained at an appropriate temperature and transferred to the laboratory, where cumulus–oocyte complexes were identified, isolated, and morphologically evaluated under a stereomicroscope. Selected oocytes were subsequently subjected to standard in vitro maturation (using CO₂ incubator), in vitro fertilization, and embryo culture procedures (under the influence of tri-gas i.e Nitrogen, Oxygen and CO₂) for subsequent assessment of embryo production. Sperm were prepared for fertilization through centrifugation in sperm prep media.

Results

The results demonstrated clear differences in ovarian activity between the two age groups. Heifers exhibited a superior follicular response compared with older cows. Seven OPU sessions were performed for each animal in both groups at the designated intervals. In the heifer group, a total of 84 and 90 ova were recovered at 14-day and 21-day intervals, respectively. The average number of follicles aspirated per OPU session was 12.00 and 12.85, respectively. In contrast, adult cows yielded considerably fewer ova, with total collections of 51 and 58 ova at the 14-day and 21-day intervals, respectively. The average follicle count per OPU session was only 7.28 and 8.28, respectively (Table – 1). These findings indicate that younger Friesian heifers possess greater ovarian reserves and higher follicular recruitment capacity than older cows. The significantly greater number of aspirated follicles in heifers ($P < 0.05$) suggests that age strongly influences ovarian responsiveness during OPU procedures.

Table - 1

Age	n	OPU		TOC		Avg of follicles per OPU		EP (No.)		EP %	
		14 D	21 D	14 D	21 D	14 D	21 D	14 D	21 D	14 D	21 D
Heifer 12 – 25 Mo	5	7	7	84	90	12.0	12.85	69	72	82.14	80.00
Cows 8 – 10 yr	5	7	7	51	58	7.28	8.28	43	42	84.31	72.41

D (Day), **EP** (Embryo production), **Mo** (Months), **n** (Number of animals, **OPU** (Ovum Pick-up), **TOC** (Total ova collected), **yr** (Years).

Increasing the interval between OPU sessions from 14 days to 21 days resulted in a modest increase in the total number of ova recovered in both age groups. In heifers, ova recovery increased from 84 to 90, while in cows it increased from 51 to 58. Similarly, the average follicle count increased slightly when the collection interval was extended. Heifers showed an increase from 12.00 follicles per session at the 14-day interval to 12.85 follicles at the 21-day interval. In cows, follicle numbers increased from 7.28 to 8.28 follicles per session. These results suggest that longer intervals between OPU sessions may provide additional time for follicular growth and recruitment, thereby improving follicular yield.

Embryo production was relatively similar between the two collection intervals in heifers. A total of 69 embryos were produced at the 14-day interval and 72 embryos at the 21-day interval. Embryo production efficiency (EP%) was 82.14% and 80.00%, respectively. Among cows, embryo production was 43 embryos at the 14-day interval and 42 embryos at the 21-day interval. Embryo production efficiency declined from 84.31% at the 14-day interval to 72.41% at the 21-day interval. Although heifers recovered substantially more oocytes than cows, embryo production efficiency remained comparable between the age groups. This observation indicates that while younger animals produce more oocytes, the developmental competence of recovered oocytes in both age groups remains relatively similar.

Statistical Analysis

Data obtained from ovum pick-up (OPU) sessions in Friesian heifers (12–25 months) and Friesian cows (8–10 years) were analyzed to evaluate the effects of donor age and collection interval (14 and 21 days) on follicular response and embryo production. Animals were maintained under identical feeding and environmental conditions to minimize confounding effects. Descriptive statistics were expressed as mean $\pm$ standard error (Mean $\pm$ SEM). Differences in the average number of follicles aspirated, total ova recovered, embryo production, and embryo production percentage (EP%) between age groups and collection intervals were assessed using a two-way analysis of variance (ANOVA), considering age group and collection interval as fixed effects. When significant differences were detected, means were compared using Tukey's post hoc test. Statistical significance was declared at $P < 0.05$.

The data gathered from the experimental observations were subjected to appropriate statistical analysis, and the results are presented in tabular form to provide a systematic and comprehensive comparison of the evaluated reproductive parameters among the experimental groups (Table – 2). The tables illustrate the observed variations in follicular activity, oocyte recovery, and subsequent embryo production in relation to donor age and OPU session interval.

Table – 2

Parameter	Heifers	Cows	Interpretation
Total ova recovered (14 days)	84	51	Higher in heifers
Total ova recovered (21 days)	90	58	Higher in heifers
Mean follicles/OPU (14 days)	12.00	7.28	Significantly greater in heifers
Mean follicles/OPU (21 days)	12.85	8.28	Significantly greater in heifers
Embryo production (14 days)	69	43	Higher in heifers
Embryo production (21 days)	72	42	Higher in heifers
EP% (14 days)	82.14%	84.31%	Comparable
EP% (21 days)	80.00%	72.41%	Slightly lower in cows

The table itself already states that the number of follicles aspirated and good-quality oocytes was significantly higher ($P < 0.05$) in heifers than in cows

Discussion

The superior ovarian response observed in heifers is likely associated with their larger ovarian reserve and greater follicular activity. Aging is known to reduce both the number and quality of ovarian follicles, which may explain the lower follicular yield in older cows. Each follicular wave is characterized by the recruitment and subsequent development of a dominant follicle. Depending on the stage of the estrous cycle, this dominant follicle may either undergo atresia or proceed to ovulation. During the luteal phase, elevated progesterone concentrations produced by the corpus luteum exert negative feedback on the hypothalamic–pituitary axis, thereby limiting the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary gland. The resulting gonadotropin concentrations are insufficient to sustain continued follicular growth and induce ovulation, leading to regression and atresia of the dominant follicle. In some cases, failure of follicular regression may result in the development of a follicular cyst. Conversely, when a follicular wave emerges during luteolysis, the decline in progesterone-mediated inhibitory effects permits increased gonadotropic stimulation. Consequently, the slight increase in ova recovery at the 21-day interval

suggests that longer recovery periods between OPU sessions allow more follicles to develop to aspiratable sizes. However, extending the collection interval did not substantially improve embryo production efficiency, indicating that factors other than follicle number, such as oocyte quality and the ovarian environment, also influence embryo development. Moreover, the efficiency of ovum pick-up (OPU) programs is generally evaluated using several reproductive and embryological parameters that reflect the success of oocyte retrieval and subsequent embryo production (Raza et.al; 2026). Boni et.al; (1997) conducted experiment on scheduled OPU twice weekly at 3- or 4-day intervals over 3 months. At 7th week, the effects of 2- and 5-day intervals between follicular punctures were tested and compared with the previous 3- and 4-day intervals. Two-day intervals resulted in a lower number of follicles than the three day interval.

According to Chaubal et al (2006), evaluation of ovum pick-up (OPU) performed once and twice weekly revealed no significant differences between the two regimens in terms of the number of follicles aspirated, oocytes recovered or blastocysts produced by day 7 of culture per cow per session. However, when assessed over a one-week period, the 2 week OPU regimen

resulted in significantly greater cumulative numbers of aspirated follicles, recovered oocytes and day-7 blastocysts compared with the 1week regimen. In contrast, further shortening the interval between successive OPU sessions to 2 days did not provide additional reproductive benefits. This more frequent schedule was associated with a reduction in the number of follicles suitable for aspiration (Boni et al., 1997) as well as the number of oocytes recovered. Nevertheless, the oocyte recovery rate and oocyte quality were comparatively higher, suggesting that although very frequent OPU may reduce the absolute yield of follicles and oocytes, it may potentially improve the efficiency and quality of the recovered oocytes. Time between OPU sessions, removal of the dominant follicle to stimulate a new wave of follicular growth, and hormonal synchronization of the reproductive cycle are techniques that ensure the optimal stage of the reproductive cycle of donor animals have already been identified by Chinarov (2024). Interestingly, despite producing fewer oocytes, older cows maintained embryo production rates comparable to those of heifers. This finding suggests that once competent oocytes are recovered, the capacity for embryo development may not differ greatly between age groups. Several important observations were evident from the experimental data. Heifers exhibited a significantly greater follicular yield and higher number of recovered oocytes compared with adult cows, indicating a more pronounced ovarian response in younger donors. Extending the interval between OPU sessions from 14 to 21 days resulted in a modest increase in follicular yield in both age groups, suggesting that a longer recovery interval may provide additional time for follicular recruitment and development. However, embryo production remained relatively stable across the two OPU intervals, indicating that increased follicular yield did not necessarily translate into a proportional increase in embryo production. Interestingly, the highest embryo production efficiency (84.31%) was recorded in adult cows subjected to the 14-day OPU interval, whereas the lowest efficiency (72.41%) was observed in adult cows collected at 21-day intervals. Overall, these findings suggest that donor age exerted a more pronounced influence on follicular and oocyte yield than on subsequent embryo developmental competence. Despite the lower follicular and oocyte yields observed in older cows, their recovered oocytes demonstrated the capacity to support embryo development at efficiencies comparable to those of younger donors. Thus, donor age and OPU interval appear to influence different

components of the OPU–IVEP process, with age being more closely associated with ovarian follicular activity, while embryo developmental competence may be influenced by additional factors beyond oocyte quantity.

Conclusion

The present study demonstrates that donor age significantly influences OPU performance in Friesian cattle. Heifers (12–25 months) exhibited significantly greater follicular activity and produced more oocytes than older cows (8–10 years). Extending the interval between OPU sessions from 14 to 21 days resulted in a modest increase in follicular yield in both age groups. Despite the higher number of recovered oocytes in heifers, embryo production efficiency remained relatively similar between heifers and cows, indicating that oocyte competence was maintained across age groups. Overall, younger donors appear to be more suitable candidates for OPU programs aimed at maximizing oocyte recovery and embryo production. The findings suggest that both donor age and collection interval should be carefully considered when designing OPU protocols to optimize reproductive efficiency in Friesian cattle.

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