FACTORS AFFECTING THE OPTIMIZATION OF OVUM PICK-UP FREQUENCY IN BRAHMAN AND FRIESIAN CATTLE DURING *IN-VITRO* FERTILIZATION; A COMPREHENSIVE REVIEW

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ABSTRACT: Reproduction is key area to produce livestock with desirable traits and in this regard, assisted reproductive technology (ARTs) play a major role particularly *in-vitro* fertilization (IVF) technology. *In-vitro* embryo production (IVEP) has increased dramatically over the past 10 to 15 years, Initially, oocytes for IVEP were collected from slaughterhouse ovaries, however, with technological advancements oocytes are now being collected from the live animal through the ovum pick-up (OPU) methodology (Sarkar D *et al.*, 2021). To maximize oocyte yield, maintain donor health and improve the efficiency of IVEP programs, it is essential to determine and optimize the appropriate frequency of OPU sessions in different cattle breeds. The optimization of ovum pick-up (OPU) frequency plays a pivotal role in maximizing oocyte recovery, ensuring donor welfare, and improving the cost-effectiveness of *in-vitro* fertilization (IVF) programs in cattle. This review consolidates current knowledge on biological, hormonal, technical, environmental, and welfare-related factors influencing OPU outcomes, with special emphasis on breed-specific differences between Bos indicus (Brahman) and Bos taurus (Friesian) cattle. Distinct ovarian physiology, including higher antral follicle counts, smaller follicle diameters, and differential follicular wave dynamics in Bos indicus, dictates a more conservative OPU frequency compared with Bos taurus donors. Evidence suggests that weekly or biweekly non-stimulated OPU is optimal for Brahman donors, while Friesian donors can sustain twice-weekly OPU under skilled management and high laboratory capacity. Hormonal priming with FSH or eCG can improve oocyte yield and competence, though repeated super stimulation demands cautious spacing (10 – 14 days) to avoid ovarian fatigue. Environmental factors such as heat stress, nutritional status and oxidative balance further modulate follicular response and oocyte quality. Despite technical advancements, significant gaps persist regarding long-term donor effects, breed-optimized FSH regimens, and frequency adaptation under tropical heat load.

Keywords: Ovum pick up, Bos indicus, Bos taurus, frequency optimization, *in-vitro* fertilization, *in-vitro* embryo production.

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INTRODUCTION

Pakistan is an agriculture country and livestock is an important sector of agriculture in Pakistan. According to the latest economic survey livestock contributes 63.6% to the agriculture's value addition and 14.97% to the country's GDP. The gross value addition of livestock sector is increased by 4.72% over the previous year. Pakistan ranks 6th in the world in terms of cattle population, maintaining around 59.7 million cattle over the past three years and producing approximately 72.34 million tonnes of cow milk and 5.96 million tonnes of beef during the same period (Pakistan Economic Survey 2024-2025). Despite being ranked 6th in the world, the nation's population has nutritional needs that cannot be met by production alone. In order to meet the needs for adopting new technologies or improving existing ones, it is necessary to produce food products in large quantities. In this case, assisted reproductive technology (ART) including *in-vitro* fertilization (IVF) can be very helpful (Sarkar D *et al.*, 2021).

Productivity is the key to growth and reproduction is backbone of animal production (Verma OP *et al.*, 2012). Reproduction is key area to produce livestock with desirable traits and here, assisted reproductive technology (ART) can play a major role including *in-vitro* fertilization (IVF) technology (Sarkar D *et al.*, 2021). It was reported that globally, 443,533 embryos were produced through *in-vitro* fertilization (IVF) in 2012, with about 80% (355,205 embryos) produced in Brazil and 17% in North America (USA and Canada) (Kaya A et al., 2018). *In-vitro* embryo production (IVEP) has increased dramatically over the past 10 to 15 years, particularly in Brazil, where more than 300,000 IVP embryos were produced in 2010. Worldwide the number of Bovine IVP embryos reached over 666,000 in 2016, of which more than 75% were produced in South America (Mapletoft RJ *et al.*, 2018).

Initially, *in-vitro* embryo production (IVEP), the oocytes were collected from the slaughterhouse ovaries and at present, oocytes are being collected from the live animal by using ovum pick-up (OPU) methodology (Sarkar D *et al.*, 2021). Even with ongoing attempts to enhance bovine *in-vitro* embryo production (IVEP), its effectiveness remains poor, as only 30 to 40% of oocytes form blastocysts following *in-vitro* maturation, fertilization, and embryo culture (Sirard MA *et al*., 2006). Despite of many benefits of *in-vitro* embryo production, the capacity to retrieve oocytes has restricted its initial use in cattle and buffaloes. These challenges have been mostly eliminated, nevertheless, by the recent advancement of low invasive ultrasound guided transvaginal oocyte retrieval (TVOR) and ovum pick up (OPU) (Verma OP *et al*., 2012). Through OPU oocytes can be aspirated from live animals. Now OPU-IVEP is used in many countries for the large scale embryo production at commercial level. This repeated recovery permits production of more embryos than might be possible by standard Embryo Transfer (ET) practice (Galli C and Lazzari G, 2008). TVOR also allows repeated collection of oocytes from endangered species of livestock or livestock of high economic importance in order to propagate such genetic resources in much faster way (Verma OP *et al*., 2012). Ovum pick-up and *in-vitro* fertilization (OPU-IVF) has emerged as a powerful tool for accelerating genetic improvement in cattle by enabling repeated collection of oocytes from elite females, thereby shortening the generation interval and increasing selection intensity. In addition, the increased embryo yield per donor per year improves the cost-effectiveness of elite animal propagation and hence the overall rate of genetic gain in a herd.

Optimizing the frequency of ovum pick-up (OPU) is essential for balancing oocyte yield, donor welfare, and the economic sustainability of *in-vitro* embryo production (IVEP) programs. Frequent OPU sessions can increase the total number of oocytes recovered per donor over time, however, excessively short intervals may compromise follicular development and oocyte competence, resulting in lower-quality embryos (De Roover R *et al.,* 2008). From an animal welfare perspective, repeated follicular aspiration at short intervals can lead to ovarian adhesions, follicular depletion, or increased stress in donor cows. Economically, each OPU session involves significant labor, hormonal costs and laboratory inputs. Therefore, identifying the optimal interval that maximizes oocyte yield while minimizing donor fatigue and procedural expenses is critical for cost-effective and sustainable genetic improvement programs (Boni R, 2012) (Dogan H *et al.,* 2024). Moreover, aligning OPU frequency with the donor's follicular dynamics and physiological condition ensures both reproductive efficiency and animal welfare, ultimately enhancing the overall productivity of OPU-IVF systems (Salek F *et al.,* 2025). Keeping in mind the growing importance of optimizing ovum pick up (OPU) frequency from live donor animals, this review article comprehensively discusses the various factors influencing the optimization of OPU procedures in two major categories of cattle, Bos indicus (e.g, Brahman) and Bos Taurus (e.g, Friezian). The discussion highlights how breed specific physiological, hormonal, technical and environmental factors affect oocyte yield, donor welfare and overall efficiency of *in-vitro* fertilization programs.

Historical Background of OPU and IVEP Basis: Initially IVEP relied on oocytes collected from ovaries obtained from slaughterhouses. This method was useful for developing the technology but not ideal for using genetically valuable live animals in commercial applications (Carter JA *et al.,* 2002). Around the mid-1980s, transvaginal ultrasound-guided aspiration was developed in humans to retrieve oocytes without surgery (Ferré LB *et al.,* 2020). Soon, this human method was adapted for animal use. In 1987, it was first implemented on cattle at the University of Utrecht in Denmark (Van Wagtendonk-de Leeuw AM *et al.,* 2006). Towards the end of the 1980s, the combination of the ultrasound-guided oocyte retrieval technique usually referred to as ultrasound-guided ovum pickup (OPU) and IVP became available. Several European artificial insemination centers implemented OPU-IVP as the primary tool to multiply genetically high merit animals. In USA the OPU-IVP technology combination was adopted on a commercial level in the late 1990s. By then, it was primarily used to produce embryos from 'problem' females that for different reasons could not generate viable embryos by conventional multiple ovulation and embryo transfer (MOET) (Ferré LB *et al.,* 2020). The table given below presents a comparison between OPU-IVP and conventional MOET techniques, highlighting the distinct advantages and technological advancements that give OPU-IVP a significant edge over traditional MOET programs.

The current technology of OPU/IVEP aims at harvesting (immature) oocytes from preselected genetically superior living cows by ultrasound guided transvaginal aspiration, followed by *in-vitro* maturation, fertilization and culture until embryos have reached the morula or blastocyst stage and can be transferred non-surgically into recipients or frozen (Van Wagtendonk-de

Leeuw AM *et al.,* 2006). OPU-IVP is now a widely used commercial technique for producing embryos from genetically superior animals, such as those from young calves or those that are unable to conceive naturally (Ferré LB *et al.,* 2023). Transvaginal ultrasound-guided oocyte retrieval (OPU) and *in-vitro* embryo production (IVEP) in cattle has shown significant progress in recent years (Ferré LB *et al.*, 2023). The combination of OPU and IVP (OPU-IVP) has been successfully and widely commercially used worldwide.

Table 1: Showing the differences between OPU-IVEP and Conventional MOET

S. No	Aspect	OPU-IVP	Conventional MOET
1	Donor condition	Performed on pregnant, non-cycling or even pre-pubertal donors	Requires cycling, non-pregnant donors with normal estrous cycle
2	Frequency	Can be repeated every 3-7 days	Done only once 45-60 days (superovulation interval)
3	Hormone use	Less or none FSH required	Requires superovulation with large FSH doses
4	Ovarian Recovery	Minimally invasive; no uterine flushing	Uterine flushing may cause inflammation or adhesions
5	Oocyte/Embryo yield	Oocytes obtain continuously from small follicles	Depends on successful ovulation and fertilization inside the donor
6	Pregnancy Status	Can be done from pregnant donors (up to 3 months)	Cannot performed on pregnant donors
7	Genetic Multiplication	Faster	Slower
8	Breed Adaptability	Suitable for Bos Indicus breeds	Works better in Bos Taurus breeds
9	Embryo production control	Lab controls fertilization and embryo culture	In vivo conditions – less control
10	Biosecurity	Reduced disease transmission	High risk of Pathogen spread
11	Cost Efficiency (long term)	Higher initial setup cost, but cheaper per embryo with continuous use	Cheaper setup, but more ostly per embryo due to hormone use and variable yield

Brief view on Transvaginal ultrasound-guided oocyte retrieval (OPU): Transvaginal ultrasound-guided ovum pick-up (OPU) is a minimally invasive technique used to recover oocytes from live donor cattle, enabling subsequent *in-vitro* fertilization (IVF) and embryo production. To aspirate cumulus-oocyte complexes (COCs) under vacuum, a transducer with a needle guide is placed vaginally, and an aspiration needle is guided into the antral and Graafian follicles using ultrasonography (Sasamoto Y *et al.,* 2003). OPU allows repeated sessions on live donors, including pregnant, lactating, or non-cycling animals, thereby broadening the donor pool (Amiridis GS *et al*., 1998). OPU has become increasingly efficient and competitive as a tool for cattle genetic programs (Boni R, 2012).

In practicality, OPU regimens differ based on the breed, program objectives, and physiological state of the donor. Hormonally stimulated OPU and non-stimulated (natural wave) OPU are two popular methods. In non-stimulated procedures, which depend on natural follicular waves, donors may have OPU at predetermined intervals (e.g., once or twice weekly) without first undergoing hormone therapy. The significance of timing within the follicular wave was demonstrated by an example research conducted on Holstein heifers, which revealed that OPU on days 2-3 or 4-7 of the cycle generated more Grade A oocytes than days 13-16 (Yesilkaya ÖF *et al.,* 2025). In order to synchronize follicular emergence and expand the pool of medium/large follicles prior to OPU, stimulated protocols require administering of gonadotropins such as FSH, GnRH and progestin (Egashira J *et al.,* 2019). The choice between non-stimulated and stimulated schedules reflects trade-offs between cost, donor stress, and yield per session.

When evaluating OPU-IVF programs, key outcome metrics include oocyte recovery rate (number of COCs recovered per follicles aspirated), oocyte quality (e.g, Grade A/B oocytes), blastocyst rate (percentage of embryos reaching blastocyst stage), pregnancy rate following embryo transfer, and donor health/long-term reproductive performance (Kharayat SK *et al.,* 2024).

Breed Physiology and Ovarian Dynamics: The ovarian morphology, follicular dynamics, and endocrine milieu of Bos indicus and Bos taurus cattle are consistently different, and these dissimilarities are relevant to OPU-IVF procedures (Silva-Santos KC *et al.,* 2014). Bos indicus females typically present a larger population of small antral follicles visible on the ovarian surface (higher surface AFC), ovulate from smaller dominant follicles, and show different patterns of follicle deviation compared with Bos taurus breeds. Additionally, under comparable circumstances, Bos indicus cows typically have higher levels of steroids and metabolic hormones (insulin, IGF-1) in the blood than Bos taurus cows (Sartori R *et al.,* 2018) (Cushman RA *et al.,* 2019).

Histological and ultrasonographic studies show that although surface antral follicle counts may be greater in Bos indicus types, the total primordial follicle reserve per gram of ovarian tissue can be lower or similar to Bos taurus breeds. This suggests that ovarian architecture differs rather than that there is a simple one-to-one reserve increase (Alward KJ *et al.,* 2023). Breed-specific responses to exogenous gonadotropins are also influenced by these combined anatomical and endocrine differences. For example, Bos indicus donors frequently exhibit higher sensitivity to gonadotropin stimulation but a more variable super ovulatory/FSH response profile than Bos taurus donors, which should be taken into account when developing stimulation protocols for OPU (Sartori R *et al.,* 2018) (Cushman RA *et al.,* 2019).

Because Bos indicus donors tend to have many small antral follicles and ovulate from relatively smaller dominant follicles, they may sustain repeated aspirations of small follicles with different recovery kinetics compared with Bos taurus donors, however, the higher surface AFC does not automatically guarantee superior oocyte competence (Silva-Santos KC *et al.,* 2014) (Cushman RA *et al.,* 2019). Although follicle size distribution, metabolic state, and aspiration timing in relation to follicular waves all affect oocyte developmental competence, high AFC females (both Bos indicus and Bos taurus) typically generate more oocytes and respond more strongly to superovulation (de Lima *et al.,* 2020) (Moon J *et al.,* 2025). Practically, this means that Bos indicus donors (Brahman) may tolerate conservative repeated non-stimulated OPU schedules (e.g, weekly) or mild stimulation better than aggressive FSH regimens used in some Bos taurus programs, while Friesian (Bos taurus) donors often respond predictably to synchronized FSH-primed protocols that allow shorter inter-OPU intervals and higher per-session yields OPU (Sartori R *et al.,* 2018) (Cushman RA *et al.,* 2019) (de Lima *et al.,* 2020). Therefore, OPU frequency optimization must be breed-specific, interval and stimulation should be matched to AFC category, follicle-size profile and metabolic/endocrine status to maximize oocyte competence and preserve long-term donor ovarian health.

Factors Influencing OPU Frequency:

1. Biological/physiological Factors:

a. **Age and Parity:** The age and parity of the donor affect the number and quality of oocytes. Compared to mature, properly conditioned donors, young pre-pubertal heifers frequently produce fewer and less developmentally competent oocytes; on the other hand, very old donors may exhibit follicular exhaustion or decreased competence. Therefore, the safe time between OPU sessions and cumulative embryo yield are influenced by age-related variations in follicular reserve and oocyte competence (Boni R *et al.,* 2012) (De Roover R *et al.,* 2008).

b. **Lactation status and Metabolic load:** When entered into intensive OPU programs, lactating cows, particularly high-yielding dairy breeds, may need longer recovery periods or nutritional support due to their altered energy balance and endocrine milieu, which can lower oocyte competence and alter follicular dynamics (negative energy balance, altered IGF-1, and altered insulin) (Siqueira LC *et al.,* 2009) (Velazquez MA *et al.,* 2023).

c. **Pregnancy Status:** OPU can be performed in early pregnancy in some protocols, but pregnancy alters progesterone levels and follicular wave dynamics; pregnant donors may show different aspiration yields and risk profiles, so pregnancy status should be considered when scheduling OPU (Manik RS *et al.,* 2003) (Gimenes LU *et al.,* 2015)

d. **Ovarian Health:** Recoverable follicle numbers and oocyte quality are decreased by prior ovarian disease such as cysts, adhesions from surgery or infection, or repeated trauma. To prevent additional damage, prolonged rest periods or the cessation of repeated aspirations may be necessary (Sasamoto Y *et al.,* 2003) (Pontes JH *et al.,* 2009).

e. **Follicular wave pattern and phase:** In contrast to random timing, aspirating during the early to mid-follicular wave (when a cohort of follicles is homogenous and medium-sized) usually improves recovery and developmental competence. Oocyte recovery rate and competence are highly dependent on the stage of the follicular wave at aspiration. Therefore, wave synchronization the timing of OPU within a recognized wave allows for more frequent and fruitful sessions without compromising quality (Gimenes LU *et al.,* 2015) (Chaubal SA *et al.,* 2007).

2. Hormonal Management:

a. **FSH Priming/ Superstimulation:** The number of medium/large follicles available for aspiration is increased when FSH is administered (frequently in conjunction with progestin priming) prior to OPU. This also usually results in an increase in oocyte yield per session and downstream blastocyst rates. Meta-analysis and controlled studies show compared to non-stimulated OPU, FSH + progestin procedures generally increase oocyte counts and embryo production, however the extent varies by breed and regimen (Sarwar Z *et al.,* 2020) (Kajaysri J *et al.,* 2024).

b. **Synchronization of Follicular wave:** GnRH, dominant follicle removal or CIDR devices are used to synchronize wave emergence so that OPU can be timed for maximal cohort homogeneity this enables shorter, predictable inter-OPU intervals in some programs (Pontes JH *et al.,* 2010) (Xiao J *et al.,* 2025).

3. Technical Factors:

a. **Operator skill and experience:** Recovery rate, oocyte quality (less trauma), and procedure time are all significantly impacted by operator expertise, competent operators achieve greater recovery rates with fewer problems, enabling safe, more frequent OPU regimens (Sasamoto Y *et al.,* 2003) (Boni R *et al.,* 2012).

b. **Ultrasound Resolution and Imaging:** Higher-resolution ultrasound reveals smaller follicles and improves targeting, increasing recovery rate. Older/low-sensitivity machines miss small follicles and decrease apparent AFC, which limits the realistic frequency because more repeated sessions become inefficient (Sasamoto Y *et al.,* 2003) (Sisodiya RK *et al.,* 2008).

c. **Needle type, Gauge and Vacuum Pressure:** Needle design (side-hole vs bevel), gauge, and aspiration vacuum settings directly influence cumulus-oocyte complex (COC) integrity and recovery rate, too high vacuum or inappropriate needle damage COCs. Optimized equipment allows safer and more frequent OPU (Boni R *et al.,* 2012) (Ferré LB *et al.,* 2023).

d. **Aspiration Technique:** Micro-techniques such as needle twisting, aspiration duration, and use of follicular flushing or media additives alter recovery and COCs quality, refined techniques reduce mechanical trauma and support more frequent collection (Boni R *et al.,* 2012) (Kaya A *et al.,* 2018).

4. Environmental and Nutritional Factors:

a. **Heat Stress and Season:** Under heat stress, it is prudent to reduce the frequency of OPU or employ compensatory cooling/nutritional interventions because elevated ambient temperatures have a deleterious impact on folliculogenesis and oocyte competence, reducing oocyte recovery, cleavage, and blastocyst rates (Miętkiewska K *et al.,* 2022) (Gómez-Guzmán *et al.,* JA 2024).

b. **Energy Balance and Body Condition Score:** Donors with low BCS or negative energy balance produce fewer competent oocytes and slower follicular recovery, maintaining a reasonable BCS (around 3/5) enhances oocyte quality and supports more frequent OPU if other factors are optimized (Bezdíček J *et al.,* 2020) (Bridges GA *et al.,* 2012).

c. **Micronutrients:** Adequate micronutrients promote follicular health, antioxidant capacity, and immune function. Deficiencies (e.g., Se, vitamin E, β-carotene) impair oocyte quality and can increase infection risk after OPU, thereby limiting feasible OPU frequency until nutritional status is corrected (Spears JW, 2000) (Gasselin M *et al.,*2020).

5. Welfare and Health:

a. **Ovarian integrity and cumulative trauma:** Repeated aspirations, particularly if done incorrectly, can result in cortical scarring, adhesions, or altered follicular architecture, to ensure long-term donor fertility, monitoring with ultrasound and recovery-allowing sessions are crucial (Ferré LB *et al.,* 2023).

b. **Infection risk and asepsis:** Transvaginal OPU is minimally invasive but breaches mucosal barriers, strict aseptic technique reduces risk of ascending infection and systemic illness, which otherwise mandates treatment and interrupts OPU schedules (Boni R *et al.,* 2012) (Gómez-Guzmán *et al.,* JA 2024).

c. **Stress, Behavior and Systemic Health:** Maximum OPU frequency should be determined by welfare considerations (sedation protocols, handling and rest times) because frequent handling, sedation/anesthesia, and the treatment itself can stress animals, impair immunity, and alter metabolic status (Naspinska R *et al.,* 2023).

6. Laboratory/IVF Factors:

a. **IVM/IVF media and culture condition:** Laboratory competence (appropriate IVM/IVF media, antioxidant supplementation, and culture systems) determines the proportion of recovered oocytes that produce transferable embryos; poor lab performance reduces the benefit of frequent OPU because more oocytes are wasted (Krisher RL *et al.,* 2024).

b. **Lab throughput and turnaround time:** How many OPUs a program can perform is limited by practical factors such as the number of oocytes a lab can process each day, incubator space, and embryologist availability. A high OPU frequency without corresponding lab throughput results in bottlenecks and decreased embryo quality (Krisher RL *et al.,* 2024).

c. **Embryo cryopreservation and transfer logistics:** The value of rapidly creating a large number of embryos is influenced by cryopreservation success rates and the availability of synchronized recipients, if transfer/recipient capacity is constrained, more frequent OPU may not result in more pregnancies and is therefore not cost-effective (Lee J *et al.,* 2024).

Evidence on optimal OPU frequencies: Numerous field and experimental investigations have compared hormonally stimulated sessions (one super stimulated OPU every 10-21 days), weekly schedules, and regular non-stimulated OPU (e.g., twice-weekly). According to a large retrospective dataset, in contrast to FSH-LH super stimulation, which produced more oocytes per stimulated session but required longer intervals between sessions and higher hormone inputs, a twice-weekly non-stimulated OPU scheme produced a high cumulative number of oocytes and IVP embryos over time, when compared over the same time span, frequent non-stimulated sessions frequently gave equal or greater cumulative embryo yield (De Roover R *et al.*, 2008). Multiple large Brazilian commercial datasets in Nelore (Bos indicus) donors reported that OPU spacing at about 15 days between sessions (often with mild control of follicular waves or targeted stimulation) produced good per-session yields and acceptable pregnancy rates after transfer. This supports intermediate intervals in Bos indicus breeds under field conditions (Pontes JH *et al.*, 2009) (Pontes JH *et al.*, 2011).

Reviews and program analysis emphasize that OPU frequency must be considered together with stimulation strategy, because stimulation increases per-session yield but changes the safe minimum recovery time (Galli C *et al.*, 2014) (Boni R, 2012). Meta-analysis of FSH + progestin priming in Bos taurus confirms that pre-OPU FSH enhances medium-sized follicle numbers, oocyte recovery and transferable embryos compared with non-stimulated OPU, but effect sizes depend on the regimen and donor category (Sarwar Z *et al.*, 2020). Overall, three useful strategies are supported by empirical data:

- Frequent non-stimulated OPU (e.g, twice weekly) for high-throughput programs with excellent lab support
- Weekly to 10 – 15 day intervals for balanced throughput and donor recovery
- FSH-primed or super stimulated cycles spaced 10 – 21 days when per-session maximization is desired

Breed Specific Findings and Practical recommended frequency ranges (Braham vs Friesian): Breed physiology has a significant influence on which of the aforementioned strategies is best. Compared to Bos taurus breeds (Friesian/Holstein), Bos indicus breeds (Brahman, Nelore) frequently exhibit deeper-lying ovaries, a propensity for varied super ovulatory responses, and distinct follicular dynamics (greater counts of small surface antral follicles but variable responsiveness to gonadotropins). Robust embryo yields and acceptable pregnancy rates were obtained in a number of sizable Nelore OPU/IVP programs when OPU was scheduled at intervals of about 15 days (or with mild stimulation and controlled wave synchronization). This suggests that Bos indicus donors can tolerate intermediate spacing better than aggressive twice-weekly stimulation schemes (Pontes JH *et al.*, 2009) (Pontes JH *et al.*, 2011).

On the other hand, when laboratory throughput and animal management permit, Bos taurus donors (Friesian/Holstein) can be incorporated into higher-frequency regimens (such as weekly or twice weekly non-stimulated, or FSH-primed shorter-interval runs) since they usually react more consistently to progestin + FSH priming (Sarwar Z *et al.*, 2020) (Galli C *et al.*, 2014). Based on the empirical evidence the following recommendations are stated in the table below:

Table 2: Showing Empirical Recommendations for optimizing OPU Frequency in Brahman and Friesian cattle

Breed	Physiological Category	Recommended OPU Frequency	Stimulation Protocol	Rationale	References
Bos Indicus	Non-stimulated donors	Every 7-15 days	None (natural wave collection)	Bos indicus breeds (Brahman) have smaller follicles and shorter follicular wave turnover; numerous aspirations run the risk of reducing donor stress and oocyte yield. Conservative frequency ensures follicular recovery and ovarian health	(Sartori R *et al.*, 2009)
Bos Indicus	FSH-primed/Super stimulated	Every 10-21 days	Mild FSH regimen (3-4 decreasing doses, 12 hrs apart)	Spacing enhances oocyte quality while preserving ovarian response over several sessions, it permits full follicular recovery and reduces fibrosis	(Sartori R *et al.*, 2009)
Bos Taurus	Non-stimulated donors	Every 7-15 days	None (natural wave collection)	Bos taurus breeds tolerate higher OPU frequency due to larger ovarian size, higher follicular count, and quicker follicle recruitment. Suitable for intensive IVP programs.	(Pontes JH *et al.*, 2011) (Merton JS *et al.*, 2003)
Bos Taurus	FSH-primed/Super stimulated	Every 10-21 days	5-6 dose FSH protocol followed by OPU 36-48 hrs after last dose	Stimulation enhances oocyte output and cohort synchronization, slightly longer intervals keep blastocyst rates high and avoid overstimulation.	(Pontes JH *et al.*, 2011) (Merton JS *et al.*, 2003)

Practical OPU-IVF Protocols and Decision logic for Brahman (Bos Indicus) and Friesian (Bos Taurus) Cattle: The brief, evidence-based procedure templates and decision logic for reasoning is provided below:

Table 3: Showing Practical OPU-IVF and decision logic for Brahman (Bos Indicus) and Friesian (Bos Taurus) Cattle

S.No	Step/ category	Description/ Protocol	Key Considerations	References
1	Donor Assessment	Evaluate breed (Bos indicus vs Bos taurus), antral follicle count (AFC), age, parity, lactational/pregnancy status, ovarian health (via ultrasound for cysts/adhesions), body condition score (BCS), and environmental stress (especially heat)	Proper donor screening ensures safe frequency setting and predicts oocyte yield potential	(Sartori R *et al.*, 2009)
2	Program Goal setting	Define whether the goal is rapid embryo production (short-term) or sustained oocyte collection (long-term)	Intensive super stimulation gives high per-session yield but lower long-term sustainability; non-stimulated frequent OPU supports throughput and donor welfare	(Pontes JH *et al.*, 2011)
3	Stimulation Strategy selection	**Non-stimulated OPU** for high-throughput systems (Bos taurus). **FSH or eCG priming** for low-frequency programs targeting high-quality embryos (Bos indicus).	Bos indicus require lower FSH or single eCG due to higher sensitivity, Bos taurus tolerate standard split-dose FSH regimens	(Pontes JH *et al.*, 2011)
4	Monitoring and feedback	Record oocyte yield, blastocyst rate, and ovarian ultrasound appearance after each session. If yield declines or ovarian lesions appear, reduce OPU frequency or pause collection	Prevents overstimulation, fibrosis, and declining reproductive performance	(Vieira LM *et al.*, 2014)
5	Sample schedule: Non-stimulated, High throughput (Typical for Bos Taurus)	**Frequency:** Twice weekly (e.g, Monday & Thursday) **Stimulation:** None **Interval:** 3 – 4 days	Suitable for Friesian cattle in commercial labs with strong IVF capacity; cost-effective, avoids hormone dependency	(Pontes JH *et al.*, 2011)
6	Sample schedule: FSH-primed (Bos taurus/Crossbred)	**Day 0:** Insert CIDR (progestin) **Days 5 – 7:** Administer 200 – 300 IU total FSH, split twice daily for 3 – 4 days **Day 8 – 9:** Remove CIDR, perform OPU 36 – 48 hrs later **Interval:** Repeat after 10 – 14 days	Increases cohort synchronization and oocyte recovery rate, ideal when aiming for maximal embryos per session	(Sirard MA *et al.*, 1999)
7	Sample schedule: Mild stimulation for Bos Indicus (Brahman/Nelore)	Use progestin + reduced total FSH (100 – 150 IU) or single low-dose eCG (200 – 300 IU). Perform OPU after 7 – 15 days depending on follicular response	Prevents overstimulation in Bos indicus breeds, allows follicular recovery and maintains oocyte competence	(Sartori R *et al.*, 2009)
8	Special Case: Pregnant Donors	OPU can be safely performed up to 90 days of gestation with reduced frequency and no super stimulation. Monitor progesterone and follicle health closely	Slightly reduced yield expected, maintain strict asepsis and ultrasound guidance	(Boni R *et al.*, 2012)
9	Supportive therapy and welfare	Analgesia and sedation (e.g, xylazine ± local lidocaine), aseptic handling, post OPU monitoring for hemorrhage or infection. Provide antioxidant supplementation (vitamin E, Se, Zn) and adequate energy nutrition	Supports welfare and recovery, enhances oocyte competence in metabolically stressed donors	(Herrera-Camacho J *et al.*, 2011)

Economic and operational considerations: OPU frequency is not only a biological question, but also an economic one. The main factors influencing cost per embryo are hormone costs (FSH/eCG), labour (operator + lab technicians), consumables (needles, media), and recipient/transfer expenses. Super stimulation raises the cost per session but may lower the cost per transferable embryo if embryo yield increases enough, on the other hand, frequent non-stimulated OPU lowers hormone costs but increases cumulative labor and lab processing load. Large-scale program analysis demonstrate that OPU/IVP can be cost-effective when lab throughput, recipient availability, and cryopreservation are well integrated: logistics (incubator space, technician time, embryo storage) frequently set the practical upper limit on the number of donors that can be aspirate (Kim D *et al.,* 2023). For programs in Bos indicus dominated regions, balancing milder stimulation with 10 – 15 day intervals has been economically favorable in many Brazilian reports because it reduced per session costs while maintaining acceptable pregnancy rates (Pontes JH *et al.,* 2009) (Pontes JH *et al.,* 2011). Finally, operator skill and lab efficiency strongly influence cost-effectiveness: experienced teams with optimized IVM/IVF protocols produce more embryos per oocyte, lowering the number of OPU sessions needed to reach genetic targets.

Gaps in Knowledge:

Long term Donor effects / repeated OPU on ovarian health: Relatively few studies have thoroughly assessed the long-term impact on donor fertility, ovarian reserve, or subtle ovarian pathology (e.g, stromal alterations, decreasing antral follicle count over time), despite the fact that many commercial programs involve repeated OPU sessions. Many technical advancements were examined in a retrospective analysis of 25 years of OPU practice, but it was also pointed out that the limits of repeated OPU remain insufficiently defined (Boni R, 2012). While large follicle numbers decreased in Bos indicus cows treated with a GnRH agonist and repeated OPU over an extended period of time, blastocyst yields remained stable, suggests resilience but also the need for further long-term data.

Breed specific optimized FSH/regimen protocols: There is increasing evidence that breed (Bos indicus vs Bos taurus) responds differently to FSH priming, superstimulation, and OPU intervals. For example, a meta-analysis in Bos taurus donors evaluated FSH + progestin effects on IVP outcomes (Sarwar Z *et al.,* 2020). Yet for Bos indicus donors, standardized FSH protocols optimized for OPU frequency are scarce. A recent study using recombinant FSH in Nelore calves (Bos indicus) explored dose-response but did not yet link to optimal inter-OPU interval or long-term donor health. (Moura *et al.,* 2025).

Heat stress/ environmental mitigation effects on OPU-IVF outcomes and frequency: While the effects of heat stress on reproduction broadly are well documented (e.g, for insemination, embryonic development) (Roth Z, 2017). However, specific data on how heat stress affects OPU frequency, oocyte recovery, oocyte competence and required rest intervals, especially in Bos indicus vs Bos taurus donors under tropical/sub-tropical conditions, remain sparse. A recent review highlighted the need for better thresholds (THI, metabolic markers) and behavioral/physiological indicators specific to OPU donors (Hendriks SJ *et al.,* 2025).

Conclusion: Ovum pick-up (OPU) combined with in-vitro embryo production (IVEP) has emerged as a transformative technology for accelerating genetic improvement and productivity in cattle. The evidence reviewed in this article demonstrates that optimizing OPU frequency is central to the biological, economic and ethical sustainability of OPU-IVF programs. OPU frequency cannot be defined as a fixed universal interval rather, it is a dynamic variable that must be tailored according to breed physiology, donor status, hormonal management, technical expertise, environmental conditions and laboratory capacity.

Clear physiological differences between Bos indicus (e.g, Brahman) and Bos taurus (e.g, Friesian/Holstein) cattle necessitate breed-specific OPU strategies. Bos indicus donors typically perform best under conservative or intermediate OPU intervals (approximately 10–15 days), often combined with mild stimulation protocols, to preserve oocyte competence and long-term ovarian health. In contrast, Bos taurus donors generally tolerate higher OPU frequencies (weekly or even twice-weekly schedules) and respond more predictably to FSH-based stimulation, making them suitable for intensive, high-throughput IVP systems. These breed-specific adaptations are essential to maximize cumulative embryo output while safeguarding donor welfare.

The review also highlights that optimal OPU frequency is shaped by multiple interacting factors, including follicular wave dynamics, age, parity, lactational and metabolic status of donors, technical proficiency of operators, ultrasound quality, aspiration parameters and aseptic standards. Environmental and nutritional influences particularly heat stress, body condition, and micronutrient status further modify both oocyte yield and quality. Importantly, laboratory efficiency and embryo culture competence determine whether increased OPU frequency translates into practical genetic and economic gains.

Despite considerable advances, significant knowledge gaps remain, particularly regarding the long-term impacts of repeated OPU on ovarian reserve, breed-specific super-stimulation protocols, and the interaction between environmental stressors and optimal OPU scheduling. Addressing these gaps through well-designed longitudinal and comparative studies will be critical for refining evidence-based guidelines.

In conclusion, optimization of OPU frequency should be approached as a breed-specific, donor-centric, and system-integrated strategy, rather than a purely procedural decision. By aligning biological limitations with technical capacity and welfare considerations, OPU-IVF programs can achieve sustainable genetic progress, improved reproductive efficiency, and enhanced productivity in both Bos indicus and Bos taurus cattle populations.

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