Impact of Follicular Aspiration on Follicular Dynamics and Ovarian Function in Dairy Cattle

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Abstract
Follicular aspiration is a widely used technique in ovum pick-up (OPU) procedures for harvesting cumulus oocyte complexes (COCs) and plays a significant role in controlling ovarian physiology in dairy cattle. Follicular development occurs in distinct waves, regulated by both endocrine and intra-ovarian mechanisms, which include follicle recruitment, selection, and dominance. When follicles, especially the dominant one, are removed through aspiration, this disrupts the natural sequence of events, leading to the emergence of a new follicular wave and changing the dynamics of follicle development. In this context, this review aims to evaluate the effects of follicular aspiration on follicular dynamics and ovarian function in dairy cattle, particularly focusing on its impact on follicular wave patterns, oocyte competence, and reproductive performance. Several factors influence the response to follicular aspiration, including the metabolic status of the dairy cattle, the stage of lactation, and intrinsic ovarian characteristics like antral follicle count. High-yielding dairy cattle may show altered responses due to endocrine imbalances related to negative energy balance, which can disrupt normal folliculogenesis. In conclusion, follicular aspiration is a valuable tool for controlling ovarian function; however, its application requires careful consideration of physiological and management-related factors.

Keywords: ovum pick-up, follicular wave emergence, oocyte recovery, dominant follicle.

1. Introduction

Follicular development in cattle is a dynamic and highly regulated process that occurs in recurrent waves during the estrous cycle. These waves are driven by the interplay between systemic endocrine signals, especially follicle-stimulating hormone (FSH), and intra-ovarian regulatory mechanisms that control recruitment, selection, and dominance of follicles [1-4]. In *Bos taurus*, the emergence of a follicular wave is closely linked to periodic rises in FSH, while the dominant follicle suppresses the growth of subordinate follicles through endocrine and local ovarian signals [1,5,6].

The process of follicular selection, also referred to as deviation, represents a critical stage in follicular dynamics, during which one follicle acquires increased sensitivity to gonadotropins and enhanced steroidogenic capacity, allowing it to become dominant [3,4]. Disruptions in follicular wave patterns may result in impaired ovulation, extended anovulatory intervals, and reduced fertility.

Follicular aspiration is a central step in ovum pick-up (OPU), a technique widely used for recovery of cumulus-oocyte complexes (COCs) from live donors for *in vitro* embryo production.

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OPU has become an essential reproductive biotechnology because it allows repeated oocyte retrieval without surgery and supports both research and commercial embryo production systems [7-11]. At the same time, follicular aspiration alters the ovarian environment, especially when dominant follicles are removed, and therefore has the potential to modify follicular wave patterns and ovarian function [3,12,13]. However, because aspiration removes growing follicles, especially dominant ones, it may also disturb the normal sequence of follicular wave emergence and influence ovarian function more broadly.

The physiological response to aspiration is not identical across animals. Lactation stage, dietary energy balance, antral follicle count, and breed-related ovarian traits can influence how the ovary responds to repeated OPU procedures and how efficiently oocytes are recovered [14-18]. In high-yielding dairy cows, negative energy balance may impair folliculogenesis and oocyte competence, which makes the interpretation of OPU outcomes more complex and reinforces the need for individualized protocols [14,19-20].

Although follicular aspiration may enhance follicular turnover and improve oocyte availability, its effects on ovarian function are complex and not fully elucidated. Repeated OPU procedures has been associated with both beneficial outcomes, such as increased follicular recruitment, and potential adverse effects, including local ovarian trauma, altered vascularization, and reduced oocyte competence.

This review aims to evaluate the effects of follicular aspiration on follicular dynamics and ovarian function in dairy cattle, with particular emphasis on follicular wave patterns, oocyte competence, and reproductive performance. Understanding how aspiration interacts with ovarian physiology is essential for optimizing reproductive technologies and improving fertility outcomes in dairy cattle [19,20].

Follicular dynamics in cattle

In *Bos taurus*, ovarian follicles develop in a cyclical manner, with successive waves of recruitment during each estrous cycle. A wave begins when a cohort of small follicles is recruited under the influence of FSH, after which one follicle is selected to become dominant and the others become subordinate and undergo atresia [1-4]. This wave system are a fundamental aspect of bovine folliculogenesis and explains why manipulation of one follicle can alter the development of the others [21,22].

Follicle selection is not determined by gonadotropins alone. It also depends on intra-ovarian mechanisms involving granulosa and theca cell function, follicular fluid composition, vascular support, and local growth factor signaling [4,5,21,22].

The dominant follicle gains a biological advantage through greater estradiol production, increased responsiveness to gonadotropins, and suppression of subordinate follicles, which together maintain dominance until ovulation or atresia [3,5,6].

In dairy cattle, ovarian dynamics are influenced by physiological status and individual follicular reserve. Follicle number is highly repeatable within animals and is inversely associated with FSH concentrations and the proportion of good-quality embryos after ovarian stimulation, indicating that ovarian phenotype is an important determinant of reproductive efficiency [15,16]. In addition, ovarian antral follicle population has been linked to embryo production performance, showing that the intrinsic ovarian environment is closely related to both follicular development and embryo output [18].

These physiological characteristics are critical for understanding follicular aspiration. When a dominant follicle is removed, the inhibitory effect on subordinate follicles is released and a new wave may emerge earlier than expected, which can temporarily reset follicular dynamics [1,12,13]. Therefore, aspiration is not only a method of oocyte recovery, but also a direct modulator of ovarian organization.

Follicular Wave Emergence

Follicular development in cattle is characterized by the emergence of two or three follicular waves during a single estrous cycle. Each wave is initiated by a temporary increase in circulating FSH concentrations, which stimulates the recruitment of a cohort of small antral follicles [1]. These follicles grow in a synchronous manner for several days until a divergence in growth rates occurs, marking the beginning of the selection process.

The number of follicles recruited and their growth potential are influenced by both endocrine and intrinsic ovarian factors. Animals with a higher

antral follicle count typically exhibit increased follicular recruitment and a greater capacity for oocyte production, whereas those with lower follicular reserves may show reduced responsiveness [15,16]. In addition, systemic factors, such as metabolic status, can modulate FSH secretion and follicular responsiveness, particularly in high-yielding dairy cows.

Selection of the Dominant Follicle

Following the recruitment phase, one follicle is selected to become dominant through a process known as deviation. This event typically occurs when the future dominant follicle reaches a diameter of approximately 8–9 mm and acquires enhanced responsiveness to LH, allowing continued growth despite declining FSH concentrations [4].

The dominant follicle produces increasing amounts of estradiol and inhibin, which suppress FSH secretion and prevent further growth of subordinate follicles [3]. This endocrine feedback mechanism ensures the selection of a single follicle for ovulation under normal physiological conditions.

Follicular Dominance and Atresia

Once established, the dominant follicle maintains its growth through LH-dependent mechanisms, while subordinate follicles undergo atresia due to insufficient gonadotropic support [2]. The duration of dominance and the fate of the dominant follicle depend on the hormonal environment, particularly circulating progesterone concentrations.

In the absence of luteolysis, the dominant follicle may undergo atresia and be replaced by a new wave. Alternatively, under appropriate endocrine conditions, it may proceed to ovulation. Disruptions in this balance, often associated with metabolic or management-related factors, can impair ovulatory function and fertility.

Follicular aspiration as an OPU component

Ultrasound-guided transvaginal follicular aspiration is the standard approach used in OPU for retrieving COCs from live cows. It is widely applied because it is minimally invasive, repeatable, and compatible with embryo production programs in dairy cows [7-10]. The technique enables recovery from antral follicles without the need for surgery, making it suitable for repeated use in genetically valuable donors [11,23].

The development of OPU has been closely linked to improvements in reproductive biotechnology. Early studies showed that transvaginal aspiration could be performed effectively with appropriate needle guidance systems, and later work expanded its use to routine embryo production and breeding programs [7-9,24]. In this context, aspiration serves both as a collection method and as a physiological intervention that influences the ovarian cycle.

Technically, OPU efficiency depends on the aspiration device, the size of the follicles targeted, aspiration pressure, and the operator's skill [8,19,20]. Because the procedure directly removes follicular structures, the timing and frequency of aspiration are important determinants of both the number of oocytes recovered and the ovarian response that follows [23,25]. For this reason, follicular aspiration should be understood as a controlled manipulation of ovarian physiology rather than a passive sampling method.

Another important point is that the developmental stage of the follicular wave at the time of aspiration strongly affects the outcome. When OPU is performed during a more favorable phase of follicular growth, more homogeneous follicles are available, and the recovered oocytes may have better developmental potential. For this reason, some authors recommend synchronizing follicular wave emergence before aspiration to improve both the quantity and quality of recovered COCs. In dairy cows, this method may be particularly beneficial since the ovarian response is often affected by metabolic and lactational stress.

Effects on follicular wave dynamics

OPU procedures can alter follicular wave dynamics by removing a growing follicle, especially the dominant follicle that normally suppresses the development of subordinate follicles. Once this inhibition is lost, a new follicular wave may emerge earlier, which indicates that aspiration can reset the temporal pattern of follicular growth [3,12,13]. This effect is one of the main reasons why aspiration is useful in reproductive management and embryo production.

The relationship between OPU procedures and follicular wave emergence is closely connected to FSH dynamics. FSH surges precede the detectable

emergence of a new follicular wave, and this hormonal rise appears to be a key signal that recruits a new cohort of follicles after dominant follicle removal [1,2,4]. Thus, OPU procedures may work by interrupting follicle dominance and allowing the FSH-driven recruitment process to proceed again.

Repeated OPU procedures can also influence the regularity of follicular turnover. Studies in cattle showed that frequent follicular aspiration may increase follicular turnover and modify subsequent follicular development, while short aspiration intervals can improve oocyte yield but do not necessarily prevent re-establishment of dominance in the next wave (8,14,20). This suggests that the ovary responds dynamically to puncture, but retains its intrinsic capacity to reorganize and re-establish follicular hierarchy. In practical terms, aspiration may be most effective when performed in a controlled hormonal and physiological context.

Synchronization of follicular waves or superovulatory protocols can be used to improve follicle availability and standardize the ovarian response before OPU [12,19,26]. Therefore, OPU procedures can be viewed as a tool that not only collects COCs, but also shapes the timing of ovarian events.

Effects on ovarian function and oocyte competence

Follicular aspiration affects ovarian function by modifying the local follicular environment and altering the endocrine balance within the ovary. Removal of follicular fluid and granulosa cells can reduce inhibin-mediated suppression and influence gonadotropin dynamics, which may help initiate the next follicular wave [1,4]. In this sense, aspiration has both a mechanical and a biological effect on ovarian function.

The impact on oocyte competence is more complex. While repeated aspiration can increase the number of recovered COCs, the developmental potential of those oocytes depends on the physiological status of the donor, the quality of the follicular environment, and the degree of follicular maturity at the time of aspiration [7,9,19]. More precisely, more oocytes do not always mean better oocytes.

Antral follicle count is particularly important in this context. Animals with higher follicle numbers often show better embryo production performance, but follicle number alone does not guarantee high oocyte quality, because the hormonal and metabolic environment also matters [15,16,18]. This explains why some donors with high follicle numbers perform better in *in vitro* embryo production (IVEP) than others with similar ovarian appearance.

In postpartum or high-yielding dairy cows, nutritional status and lactational stress may further impair ovarian physiology. Negative energy balance can alter progesterone concentrations, follicular dynamics, and transcriptional patterns, which in turn may reduce the competence of retrieved oocytes [14,15,19]. Therefore, follicular aspiration should be interpreted in the context of the animal's metabolic state, not only as a technical procedure.

Although follicular aspiration is considered a minimally invasive procedure, repeated puncture of the ovarian surface may induce local tissue alterations. These include microtrauma, hemorrhage, and inflammatory responses, which can affect the ovarian microenvironment. Changes in vascularization and local signaling pathways may influence subsequent follicular development and oocyte competence.

The extent of these effects is influenced by technical factors such as needle size, aspiration pressure, and operator experience. Proper technique and appropriate intervals between procedures are therefore essential to minimize potential damage and maintain ovarian function.

Factors influencing the response to follicular aspiration

The ovarian response to aspiration varies according to donor characteristics and management conditions. Stage of lactation and dietary energy level are important because they influence folliculogenesis and the way the ovary responds to repeated OPU procedures [14]. In dairy cows, especially those with high milk yield, negative energy balance may reduce the efficiency of follicular development and compromise the quality of recovered oocytes [14,19].

Antral follicle count is another major factor. Follicle numbers are highly repeatable within individual animals and are associated with ovarian stimulation outcomes, embryo quality, and embryo production efficiency [16,18]. This suggests that cows differ intrinsically in their

ovarian reserve and in their expected response to aspiration, which supports the value of donor selection before OPU.

Breed-related differences also influence ovarian behavior. *Bos indicus* cattle have distinct reproductive cycles and follicular dynamics compared with *Bos taurus* cattle, which means that protocols cannot be transferred directly across populations without adaptation [13,17]. This is especially relevant when comparing studies or applying OPU in crossbred or tropical cattle systems.

Technical variables are equally important. Needle design, aspiration frequency, and the timing of repeated COCs collections all affect recovery and ovarian response [8,20,23]. Superstimulation may also be used to increase embryo production in selected cows, but the response depends on individual ovarian physiology and the adapted protocol used [24,26].

Practical implications for dairy cattle reproduction

Follicular aspiration is valuable as it facilitates repeated OPU procedures for COCs recovery, *in vitro* embryo production, and breeding programs.

This makes OPU especially useful in genetically superior donor cows, where repeated use of the same donor can accelerate genetic progress and improve reproductive efficiency [7,10,11,14].

One important advantage is that aspiration can be scheduled at relatively short intervals without fully suppressing ovarian activity. Repeated OPU procedures may promote follicular turnover and provide a more predictable source of oocytes, particularly when combined with wave synchronization or adapted superovulation protocols [12,19,26]. However, the goal is not only quantity, but also developmental competence of the recovered oocytes.

In some studies, twice-weekly aspiration produced more follicles and more COCs per session than once-weekly aspiration, supporting the idea that more frequent follicular aspiration can maintain a more dynamic ovarian environment. However, excessive manipulation may also contribute to local tissue disturbance and reduced efficiency over time. Additional gonadotropin treatment does not always improve oocyte yield, which suggests that response depends more on donor status and protocol design than on stimulation alone. For this reason, repeated OPU procedures should be managed carefully to maintain both output and ovarian health. These benefits must be interpreted cautiously because the optimal interval may vary with breed, ovarian status, and physiological stage.

The practical success of OPU procedures depends on matching the protocol to the donor. Cows with favorable antral follicle counts, better metabolic status, and a stable physiological state are more likely to yield good-quality oocytes and better embryo production outcomes [15,16,18]. In contrast, cows under metabolic stress or during an unfavorable stage of lactation may respond less efficiently to aspiration [14,19].

Thus, follicular aspiration is best viewed as part of an individualized reproductive strategy rather than a uniform collection procedure. When timed and applied correctly, it can improve oocyte recovery, support embryo production, and help manage ovarian function in dairy cows [20,23].

Conclusion

Follicular aspiration is a valuable tool in dairy cattle reproduction because it influences both follicular dynamics and ovarian function. By removing the dominant follicle and modifying intra-ovarian signaling, it can trigger new follicular wave emergence and support repeated COCs recovery for *in vitro* embryo production [3,7, 12, 14].

Its effectiveness, however, depends on donor physiology, ovarian reserve, lactation stage, energy balance, and technical protocol [14-16,19]. Therefore, the most effective use of follicular aspiration in dairy cows requires individualized management and a clear understanding of follicular physiology.

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