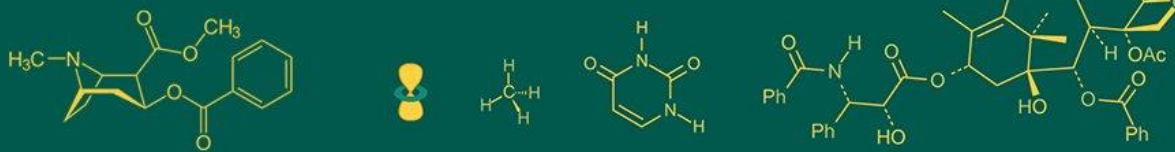


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Recent advances in reproductive physiology and their applications in dairy animals

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Abstract

Reproductive efficiency remains a critical determinant of profitability and sustainability in dairy production systems worldwide. Over the last two decades, substantial advances in reproductive physiology have transformed our understanding of folliculogenesis, oocyte competence, endocrine regulation, embryo-maternal communication, and reproductive immunology. The integration of physiological knowledge with emerging technologies such as genomics, transcriptomics, metabolomics, artificial intelligence (AI), embryo biotechnology and gene editing has revolutionized reproductive management in dairy cattle and buffaloes. These innovations have improved fertility monitoring, genetic selection, embryo production, and disease resistance while reducing reproductive wastage. This review critically examines recent advances in reproductive physiology and their practical applications in dairy animals, highlighting current challenges and future research directions.

Keywords: Reproductive physiology, Kisspeptin, embryo transfer, embryo splitting, CRISPR

1. Introduction

Reproductive performance directly influences milk production, genetic progress, and farm profitability. In high-producing dairy animals, reproductive efficiency has declined due to increased metabolic demands, environmental stress, and management-related factors. Traditional reproductive management approaches often fail to address complex interactions among endocrine, metabolic, immune, and genetic factors affecting fertility. Recent advances in reproductive physiology have enabled a systems-level understanding of reproductive processes. Integration of reproductive endocrinology, molecular biology, bioinformatics, and precision technologies has provided novel tools for improving fertility and reproductive management (Wiltbank *et al.*, 2022; Ribeiro *et al.*, 2023) ^[28, 21].

2. Emerging Concepts in Reproductive Physiology

2.1 Neuroendocrine Regulation of Reproduction

Recent studies have identified the critical role of Kisspeptin-GnRH neurons in regulating reproductive function. The neuroendocrine system plays a central role in regulating reproductive function through coordinated interactions between the hypothalamus, pituitary gland, and gonads, collectively known as the Hypothalamic-Pituitary-Gonadal (HPG) axis. Recent advances in reproductive physiology have identified kisspeptin neurons as the master regulators of reproductive function. These neurons act upstream of gonadotropin-releasing hormone (GnRH) neurons and are essential for the initiation of puberty, maintenance of estrous cyclicity, and fertility (Pinilla *et al.*, 2012; Smith, 2018) ^[20, 26].

Kisspeptin is a peptide encoded by the KISS1 gene and exerts its effects through the G-protein coupled receptor KISS1R (GPR54). Kisspeptin neurons are primarily located in the arcuate nucleus (ARC) and preoptic area of the hypothalamus. They integrate signals from metabolic, environmental, and hormonal pathways and stimulate the secretion of GnRH. GnRH subsequently acts on the anterior pituitary gland to induce the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which regulate follicular growth, ovulation, and corpus luteum formation (Oakley *et al.*, 2009; Pinilla *et al.*, 2012) ^[18, 20].

Recent studies have also identified a specialized population of neurons known as KNDy neurons, which co-express Kisspeptin, Neurokinin B (NKB), and Dynorphin (Dyn). These neurons regulate the pulsatile secretion of GnRH. Neurokinin B stimulates GnRH pulse

generation, whereas dynorphin suppresses GnRH release, creating a rhythmic pattern of gonadotropin secretion necessary for normal reproductive function (Lehman *et al.*, 2010) [11].

Furthermore, reproductive activity is closely linked to the animal's metabolic status. Hormones such as leptin, insulin, and insulin-like growth factor-1 (IGF-1) influence kisspeptin neurons, thereby connecting nutritional status with reproductive performance. Negative energy balance, commonly observed in high-producing dairy cows during early lactation, suppresses kisspeptin signaling, resulting in delayed ovulation and reduced fertility (Lucy *et al.*, 2014) [13].

2.1.1 Significance in Dairy Animals

Understanding kisspeptin-GnRH signaling has important implications for improving reproductive efficiency in dairy cattle and buffaloes. Manipulation of this pathway offers potential strategies for:

- Induction of puberty in delayed-maturing animals.
- Treatment of postpartum anestrus.
- Improvement of ovulation rates.
- Enhancement of fertility in repeat breeders.
- Development of novel reproductive therapeutics.

Research on kisspeptin administration in cattle has demonstrated increased LH secretion and stimulation of ovulation, suggesting potential applications in estrus synchronization and reproductive management programs.

2.2 Metabolic-Reproductive Interactions

The relationship between metabolism and reproduction is a fundamental aspect of reproductive physiology in dairy animals. Reproductive processes require a substantial supply of energy and nutrients; therefore, metabolic status strongly influences ovarian activity, estrous cyclicity, conception rate, embryo survival, and overall fertility. In modern high-producing dairy cows, the metabolic demands of lactation often exceed dietary energy intake during the early postpartum period, leading to a state known as Negative Energy Balance (NEB). This condition is recognized as one of the major causes of reduced reproductive efficiency in dairy herds (Butler, 2021; Lucy *et al.*, 2014; Wiltbank *et al.*, 2022) [3, 13, 28].

Recent advances in reproductive physiology have revealed that reproductive function is closely regulated by metabolic hormones and nutrient-sensing pathways that link energy status with the hypothalamic-pituitary-gonadal (HPG) axis. Hormones such as insulin, insulin-like growth factor-1 (IGF-1), leptin, growth hormone (GH), and ghrelin act as metabolic signals that influence reproductive performance (Diskin and Kenny, 2016; Leroy *et al.*, 2008) [6, 12].

2.2.1 Physiological Basis of Metabolic-Reproductive Interactions

During early lactation, dairy cows experience increased energy demands for milk production. Because feed intake cannot immediately meet these demands, cows mobilize body fat reserves, resulting in:

- Increased non-esterified fatty acids (NEFA).
- Elevated β -hydroxybutyrate (BHBA) concentrations.
- Reduced blood glucose levels.

- Decreased insulin and IGF-1 concentrations.

These metabolic changes affect reproductive tissues and endocrine pathways, leading to impaired fertility (Butler, 2021; Leroy *et al.*, 2008; Wiltbank *et al.*, 2022) [3, 12, 28].

2.3 Estrus synchronization

Estrus synchronization is a reproductive management technique that allows a group of females within a herd to exhibit estrus and ovulate within a predetermined time frame, thereby facilitating planned breeding and improving reproductive efficiency. The development of estrus synchronization protocols has significantly enhanced the effectiveness of dairy breeding programs and has become an integral component of reproductive management in modern dairy herds (Macmillan, 2010; Channo *et al.*, 2021) [14, 4].

The widespread adoption of synchronization programs is primarily attributed to their ability to facilitate Timed Artificial Insemination (TAI), thereby reducing reliance on visual estrus detection. Estrus detection remains a major challenge in high-producing dairy cattle because of reduced estrous expression, increasing herd size, labor constraints, and the subjective nature of behavioral observations. Timed breeding protocols overcome these limitations by allowing insemination at predetermined times without the need for estrus detection (Macmillan, 2010; Ribeiro *et al.*, 2023) [14, 21].

The principal hormones utilized in estrus synchronization programs include prostaglandin $F_{2\alpha}$ (PGF $_{2\alpha}$), gonadotropin-releasing hormone (GnRH), and progestins, which regulate luteolysis, follicular development, ovulation, and estrous behavior. Common synchronization protocols include Select Synch, Ovsynch, Cosynch, CIDR-based programs, and MGA-PGF $_{2\alpha}$ systems. Among these, heifers respond particularly well to the Melengestrol Acetate (MGA)-PGF $_{2\alpha}$ protocol, which has demonstrated high estrus response and pregnancy rates (Amare and Ayalew, 2021) [1].

A major limitation of conventional synchronization programs is their reduced effectiveness in non-cycling animals, including prepubertal heifers and postpartum anestrus cows. However, recent advancements have focused on incorporating progesterone supplementation and resynchronization strategies to improve fertility in these animals. Modern resynchronization protocols permit early identification and reinsemination of non-pregnant cows following pregnancy diagnosis by ultrasonography, thereby reducing the interval between inseminations and improving overall reproductive performance (Macmillan, 2010; Wiltbank *et al.*, 2022) [14, 28].

Recent developments in precision reproductive management have further enhanced synchronization efficiency through integration with ultrasonography, activity monitoring systems, automated progesterone measurement, and artificial intelligence-based decision-support tools. These innovations improve breeding accuracy, increase pregnancy rates, and reduce reproductive losses (Bewley, 2024; Ribeiro *et al.*, 2023) [2, 21].

Overall, advances in estrus synchronization have substantially improved reproductive efficiency, genetic progress, and economic returns in dairy production systems by reducing labor requirements, increasing service rates, shortening calving intervals, and enhancing herd fertility (Wiltbank *et al.*, 2022) [28].

2.4 Sex-Sorted Semen Technology

Sex-sorted semen technology represents one of the most significant innovations in modern dairy cattle breeding. The technology enables the separation of X chromosome from Y chromosome, allowing producers to predetermine the sex of offspring with approximately 85-95% accuracy. Since female calves are preferred in dairy farming for herd replacement and milk production, sex-sorted semen has become an important tool for accelerating genetic gain and improving reproductive efficiency (Seidel, 2020) ^[25].

The most widely used method for sperm sexing is flow cytometric sorting, which exploits the difference in DNA content between X-bearing and Y-bearing sperm cells. In cattle, X-bearing sperm contain approximately 3.8-4.0% more DNA than Y-bearing sperm. Sperm cells are stained with a fluorescent DNA-binding dye, and individual sperm are sorted according to fluorescence intensity using high-speed flow cytometry (Seidel, 2014) ^[24].

2.4.1 Recent Technological Advancements

Recent improvements in sex-sorting technology have addressed earlier limitations related to reduced fertility and sperm damage. These advancements include:

- High-speed flow cytometers with improved sorting accuracy.
- Reduced mechanical and oxidative stress during sperm processing.
- Enhanced semen extenders and cryopreservation protocols.
- Development of SexedULTRA™ and similar technologies with improved conception rates.
- Integration of genomic selection with sexed semen programs.

These innovations have increased conception rates from approximately 70-80% of conventional semen fertility to nearly 85-90% under optimal management conditions (DeJarnette *et al.*, 2021) ^[5].

2.5 Embryo Transfer and Multiple Ovulation and Embryo Transfer (MOET)

Embryo Transfer (ET) is one of the most successful reproductive biotechnologies used for accelerating genetic improvement in dairy animals. The technique involves the collection of embryos from genetically superior donor females and their transfer into recipient females of lower genetic merit. This approach enables a single elite female to produce a substantially greater number of offspring during her reproductive lifetime than would be possible through natural reproduction, thereby increasing the rate of genetic gain and dissemination of superior germplasm (Hasler, 2014; Mapletoft and Bó, 2016) ^[8, 15].

Multiple Ovulation and Embryo Transfer (MOET) is an advanced embryo transfer program in which donor animals are hormonally stimulated to produce multiple ovulations using exogenous gonadotropins, particularly PMSG and FSH. Following superovulation, donors are inseminated, and embryos are collected non-surgically from the uterus approximately 6-8 days after estrus. Viable embryos are subsequently transferred to synchronized recipient females or cryopreserved for future use (Mapletoft and Bó, 2016; Pereira *et al.*, 2022) ^[15, 19].

2.5.1 Physiological Basis of MOET

Under normal physiological conditions, a cow typically ovulates a single dominant follicle during each estrous cycle. In MOET programs, administration of exogenous FSH or PMSG stimulates the growth and ovulation of multiple follicles simultaneously, resulting in the production of several transferable embryos from a single breeding cycle. The efficiency of MOET is influenced by numerous physiological factors, including ovarian follicular reserve, endocrine regulation of follicular growth, superovulatory response, fertilization efficiency, embryo quality, and the degree of synchronization between donor and recipient animals (Mapletoft *et al.*, 2023) ^[16].

2.5.2 The success of MOET depends upon

- Ovarian follicular reserve.
- Endocrine regulation of follicular growth.
- Superovulatory response.
- Fertilization efficiency.
- Embryo quality.
- Synchronization between donor and recipient animals.

Recent advances in understanding follicular wave dynamics and the application of Anti-Müllerian Hormone (AMH) as a biomarker of ovarian reserve have significantly improved donor selection and prediction of superovulatory response. Animals with higher circulating AMH concentrations generally produce more follicles and transferable embryos, thereby enhancing the efficiency of MOET programs (Ireland *et al.*, 2011; Ribeiro *et al.*, 2023) ^[10, 21].

Furthermore, integration of MOET with genomic selection has revolutionized dairy cattle breeding by enabling the identification and multiplication of genetically superior females at an early age. The combination of MOET, embryo cryopreservation, and genomic evaluation has substantially accelerated genetic progress while reducing generation intervals in dairy populations (Hayes *et al.*, 2009; Hansen, 2020) ^[9, 7].

2.6 Embryo splitting

Embryo splitting is a micromanipulation technique in which an early-stage embryo is artificially divided into two or more parts to produce genetically identical embryos. The resulting embryos can continue normal development after separation and may be transferred to synchronized recipient females. This technology mimics the natural process of monozygotic twinning and has been explored as a means of increasing the reproductive output of genetically superior animals (Said *et al.*, 2020) ^[23]. Typically, embryos at the morula or blastocyst stage are bisected using micromanipulation equipment under an inverted microscope, producing identical demi-embryos capable of further development.

Embryo splitting has important applications in dairy cattle breeding programs because it increases the number of offspring produced from elite donor animals, thereby enhancing the rate of genetic improvement. The integration of embryo splitting with embryo transfer, genomic selection, and advanced reproductive technologies offers opportunities for accelerating genetic gain, improving breeding efficiency, and facilitating research involving genetically identical animals (Mapletoft *et al.*, 2023) ^[16]. Furthermore, embryo manipulation technologies have contributed significantly to modern livestock biotechnology by supporting the

production of monozygotic twins, transgenic animals, and other advanced reproductive applications (Hansen, 2020) ^[7].

2.7 Gene Editing and Genome Engineering

Gene editing and genome engineering represent some of the most revolutionary advances in modern reproductive physiology and animal biotechnology. These technologies enable precise modification of specific DNA sequences within the genome, allowing scientists to introduce, delete, or alter genetic material associated with desirable traits. Among various genome-editing tools, the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated protein 9 (CRISPR-Cas9) system has emerged as the most efficient, economical, and versatile technique for targeted genome modification (Van Eenennaam, 2021) ^[27].

CRISPR-Cas9 functions as a molecular "genetic scissors" that uses a guide RNA (gRNA) to recognize a specific DNA sequence and direct the Cas9 nuclease to induce a double-stranded break at the target site. The cell subsequently repairs the break through non-homologous end joining (NHEJ) or homology-directed repair (HDR), enabling precise genetic modifications.

2.7.1 Significance in Reproductive Physiology

Genome editing has created new opportunities for understanding and manipulating genes involved in:

- Puberty onset.
- Fertility and fecundity.
- Embryonic development.
- Gametogenesis.
- Maternal recognition of pregnancy.
- Disease resistance.
- Heat stress adaptation.

By modifying reproductive genes, researchers can investigate their physiological functions and develop animals with superior reproductive performance. In dairy cattle, genome editing has been explored for improving fertility, enhancing resistance to infectious diseases such as mastitis, increasing heat tolerance, and accelerating genetic gain through the introduction of favorable alleles into elite breeding populations (Mueller *et al.*, 2022; Van Eenennaam, 2021) ^[17, 27].

The integration of CRISPR-Cas9 with reproductive biotechnologies such as embryo transfer, *in vitro* fertilization (IVF), and genomic selection is expected to play a major role in the development of next-generation dairy animals with improved productivity, adaptability, and reproductive efficiency (Hansen, 2020) ^[7].

3. Conclusion

Recent advances in reproductive physiology have substantially improved our understanding of the complex interactions among endocrine, metabolic, genetic, and environmental factors regulating fertility in dairy animals. Discoveries related to kisspeptin-GnRH signaling, metabolic regulation of reproduction, follicular dynamics, reproductive immunology, and embryo-maternal communication have provided new insights into the mechanisms controlling reproductive function. Simultaneously, the integration of reproductive physiology with advanced biotechnologies such as estrus synchronization, fixed-time artificial insemination (FTAI), sex-sorted semen, embryo transfer

(ET), multiple ovulation and embryo transfer (MOET), embryo splitting, genomic selection and CRISPR-Cas9-based genome engineering has transformed reproductive management in dairy cattle and buffaloes.

Despite significant progress, challenges such as declining fertility in high-producing dairy cows, heat stress, embryonic mortality, high implementation costs, and regulatory concerns regarding gene editing remain important constraints. Future research should focus on integrating multi-omics technologies, precision nutrition, artificial intelligence, and genome engineering to develop sustainable, climate-resilient, and highly productive dairy production systems.

Overall, recent advances in reproductive physiology have revolutionized dairy animal reproduction and will continue to play a pivotal role in enhancing reproductive efficiency, accelerating genetic improvement, and ensuring sustainable dairy production in the future.

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