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Review Article

Comprehensive Hormonal Profiling in Post-partum Dairy Buffaloes: Insights, Challenges, and Future Perspectives

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ABSTRACT

This review paper aims to provide a comprehensive overview of hormonal profiling in post-partum dairy buffaloes, elucidating its significance in reproductive management, milk production, and overall herd health. We discuss the hormonal changes occurring during the post-partum period, factors influencing these changes, current methods of hormonal assessment, and their applications in reproductive and productive efficiency. Furthermore, we address challenges and gaps in existing research and propose future directions for advancing hormonal profiling techniques in dairy buffalo management.

Keywords: Dairy buffalo, oestradiol, hormone profiling, post-partum, progesterone

INTRODUCTION

Importance of the Post-partum Period in Dairy Buffalo

The buffalo has earned the nickname "The Black Gold" due to its high worth and potential for growth and progress (Bilal et al., 2006). The buffalo *(Bubalus bubalis)* exhibits a reproductive cycle influenced by the seasons. During the late summer and early autumn, their sexual activity is triggered by the reduction in daylight hours (short days), as stated by Sethi et al. (2021). Their reproductive success heavily influences the profitability of dairy herds. Reproductive efficiency directly impacts the milk each cow produces during their time in the herd. It, in turn, affects the profitability and lifespan of the cow within the herd.

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The post-partum phase is crucial in animal reproduction, and its length affects reproductive effectiveness. Following parturition, it is typical for an animal to experience a lack of cyclicity. However, if this time persists for an extended duration, it becomes problematic and diminishes the animal's reproductive effectiveness (Sethi et al., 2021). Animals experience a delay in returning to their normal reproductive cycle due to low energy levels, depleted body reserves, and post-partum diseases. The typical process of follicular growth involves the stages of follicular recruitment, deviation, selection, growth, and ovulation. If any of these processes fail, the post-partum period will be prolonged.

Overview of Hormonal Changes During the Post-partum Period

Post-partum buffaloes experience hormonal changes, specifically in oestradiol (E2) and progesterone (P4) levels, which affect the quality of oocytes and influence reproductive stages, including oestrous, anoestrous, and cystic pathologies (Kumar et al., 2015). In Jaffarabadi buffaloes, the level of progesterone in the blood decreases before giving birth and reaches its highest point on the day of calving. In contrast, cortisol levels are highest when giving birth, and Prostaglandin F2α Metabolite (PGFM) dramatically increases after parturition, as stated by Dhami et al. (2019). The initial post-partum phase can result in a negative energy balance (NEB) because of metabolic stress generated by reduced feed intake of dry matter and heightened energy requirements during calving and the onset of lactation.

Significant variations in E2 concentrations have been observed in serum and follicular fluid, suggesting ongoing follicular development and regression in anoestrous animals. The reduced E2 levels in cystic animals are likely due to insufficient luteinizing hormone (LH) levels (Wathes et al., 2007). Reported P4 levels align with existing literature, showing a decrease during oestrous (Honparkhe et al., 2008). Dhami et al. (2019) documented a notable decline in plasma P4 levels and a simultaneous increase in E2 levels during calving. Subsequently, both hormones decreased in the early post-partum period, followed by an increase between days 30–60 post-partum, coinciding with the return of ovarian follicles and corpus luteum (CL) formation. Additionally, Dhami et al. (2019) observed a significant rise in plasma cortisol levels on the calving day compared to seven days before and after, indicating heightened stress during calving.

This review paper aims to determine and study the hormones involved, the changes in hormones, and the factors influencing hormonal profiles in post-partum dairy buffaloes. It will also provide strategies and future directions in the hormonal profiling of dairy buffaloes.

HORMONAL INVOLVED IN POST-PARTUM DAIRY BUFFALOES

Ovarian Hormone (Progesterone)

Progestogens are a class of hormones with comparable physiological functions, with P4 being the most significant. P4 regulates the oestrous cycles. Studies by Ahmad et al.

(1977), Mondal and Prakash (2003), and Mondal et al. (2003) have consistently found that the peripheral plasma P4 profile in buffalo closely resembles that of cattle. The study conducted by Saqib et al. (2021) demonstrated that P4 significantly impacts metabolic status, stress levels, milk supply, and reproductive cyclicity. During the post-partum period, there is a change in P4 levels. Ahmad et al. (1977) stated that P4 levels exhibit synchronous fluctuations with the expansion and regression of CL in cycling buffalo because CL is the origin of P4. Batra et al. (1979) reported that P4 levels regularly increase in animals who successfully conceive but fall three days before the second oestrous in those who fail to conceive. The decrease in P4 levels varies depending on when the CL regresses.

Besides that, according to Srivastava et al. (1999), the peripheral plasma P4 levels can vary throughout the seasons. In hotter months, P4 levels are lower during oestrous, and the mid-luteal phases compared to cooler months. Rao and Pandey (1982) propose that lower P4 levels during summer lead to reduced oestrous expression and lower conception rates. Terzano et al. (2012) discovered a substantial increase in P4 levels throughout the summer in comparison to winter. Extended exposure to high temperatures might increase the levels of P4 in the peripheral plasma because of the activity of the adrenal cortex caused by stress (Abilay et al., 1975). Furthermore, P4 levels may differ depending on an individual's nutritional state (Ronchi et al., 2001). Insufficient nourishment in buffaloes, compounded by elevated ambient temperatures, may result in an extended period of reproductive dormancy.

Ovarian Hormone (Oestradiol)

Oestrogens, particularly E2, are endogenous hormones synthesized by the ovary and conveyed by carrier proteins. They influence the central nervous system, resulting in the manifestation of oestrous behaviour in females. The E2 profile in buffalo peripheral plasma is comparable to that in cattle. Riverine and swamp buffaloes have similar E2 profiles during the oestrous cycle (Mondal et al., 2006). The higher levels of E2 in milk compared to plasma have led to an investigation of whether the mammary gland is responsible for absorbing E2 or if the hormone is partially produced inside the mammary tissue. The levels of E2 in the ovarian follicular fluid are markedly higher than in the peripheral circulation (Palta et al., 1998). There is a positive correlation between the E2 levels and the size of the follicle. A study by Palta et al. (1998) found that the level of E2 in large follicles is considerably greater than in medium follicles, whereas medium follicles have higher E2 levels than small follicles. However, Parmar and Mehta (1994) found that the medium-sized follicles had considerably elevated levels of E2 compared to small and large follicles.

Weather conditions also influence the level of E2 plasma (Ronchi et al., 2001). A study by Rao and Pandey (1983) revealed that E2 levels were lower during the summer months compared to the colder months when examining several seasons (hot-dry, hot-humid, warm and cold). Rao and Pandey (1982) determined that the main reason for a higher occurrence of silent oestrous during the summer is the decreased peak levels of E2 during oestrous, along with decreasing levels of P4.

Pituitary Hormone (Prolactin)

The rise in peripheral plasma prolactin levels during the summer season, in contrast to the winter season, has been linked to the impact of photoperiod on the functioning of prolactin in the pineal glands, as shown by various studies (Mondal et al., 2006). Besides photoperiod, the surrounding temperature significantly affects the prolactin levels. The diurnal fluctuations in prolactin levels were more obvious in summer than in winter, mostly because of the greater difference in ambient temperature between morning, noon, evening, and night. Increased prolactin levels during the summer seasons are believed to interfere with the reproduction cycle and fertility. The reason for this is that prolactin has a direct effect on the synthesis of ovarian steroids by altering the quantity of LH receptors. Besides that, it also obstructs the regular release of LH from the brain and prevents the beneficial effects of oestrogen on LH secretions (Mondal et al., 2006). Singh and Madan (1993) demonstrated that lactation influenced prolactin levels. It was noted that non-lactating buffaloes had higher levels of prolactin throughout the summer compared to lactating ones and vice versa during winter. Furthermore, observations have shown that parity influences peripheral prolactin levels, with multiparous buffaloes exhibiting higher levels on the day of oestrous compared to primiparous buffaloes. Research by Mondal et al. (2006) demonstrated that the quantities of prolactin in milk are more substantial compared to those in plasma, suggesting a possible transfer of prolactin from plasma to milk.

Pituitary Hormone (Follicle Stimulating Hormone)

Follicle-stimulating hormone (FSH) promotes the follicle's development and oestrogen production by granulosa cells in the ovarian follicles. The highest levels of FSH coincided with LH and lasted six to nine hours (Seren et al., 1994). Nevertheless, they noticed that the highest peak of FSH occurs on the tenth day after oestrous, and the last increase is shown on the fourth and fifteenth days after oestrous. After the simultaneous increase of FSH and LH before ovulation, LH levels quickly return to their original levels, and FSH levels gradually decline (Kaker et al., 1980). It has been noted that a reduction in the secretion of LH is associated with the cessation of dominance and the end of the follicular wave, resulting in the absence of ovulation. Weather conditions also influence FSH levels (Prakash et al., 1997). Studies by Janakiraman et al. (1980) have demonstrated that levels of FSH are significantly higher during the oestrous and luteal phases in the peak breeding seasons in Surti buffaloes compared to the equivalent phases in the medium and low breeding seasons.

Pituitary Hormone (Luteinizing Hormone)

LH is essential for evaluating ovarian activity, as its surge prior to ovulation is responsible for the follicular wall's rupture and the egg's release. The radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA) technique has been used to clearly identify a pre-ovulatory LH increase in buffalo. Like cattle (Rahe et al., 1980), the LH levels in the peripheral blood stay normal for the whole oestrous cycle. The time interval between the highest point of E2 hormone and the highest point of LH hormone has been recorded to be 14–15 hours. However, the duration of LH peak has been estimated to be around 6–12 hours in Mediterranean Italian buffaloes (Seren et al., 1994). Besides that, Terzano et al. (2012) reported an 8-hour interval between the LH surge and the onset of oestrous.

Like other animals, buffalo have changes in peripheral LH levels due to seasonal variations. Rao and Pandey (1983b) showed that LH levels peak on the day of oestrous during cooler months as opposed to hotter months. The variation in LH production in response to E2 can be explained by the failure of the hypothalamohypophyseal axis to consistently enhance LH secretion. Terzano et al. (2012) found that the frequency and amplitude of pulses during the follicular phase exhibit a considerable increase in winter compared to summer seasons. Terzano et al. (2012) discovered that in the summer, the absence of ovarian activity and the lack of pre-ovulatory surge are linked to reduced levels of basal LH. However, Kaker et al. (1980) stated that the average peak of LH levels during ovulation was similar in hot and cool months. The occurrence of silent oestrous during summer can be ascribed to the reduction in the maximum level of LH and the decrease in the P4 level (Rao & Pandey, 1982).

Thyroid Hormone

Thyroid hormones (TH) are involved in the complex hormonal process that regulates steroidogenesis in the ovary. Jorritsma et al. (2003) revealed a correlation between lower triiodothyronine (T3) levels, reduced E2 levels, and decreased oestrous expression. Female animals may experience reproductive issues because of abnormal thyroid function. According to Mutinati et al. (2010), TH directly impacts ovarian function in cattle by influencing the activity of granulosa and thecal cells. Additionally, TH has a substantial effect on the growth of embryos in cattle before and after implantation. Keisler and Lucy (1996) found that artificially induced hypothyroidism negatively affects the rate of conception in dairy cattle. A study by Ghuman et al. (2011) found that even a small amount of thyroid activity in lactating buffaloes can impact their fertility.

Adrenal Hormone (Cortisol)

Cortisol quantifies the overall stress levels in animals (Sampath et al., 2004). The decrease in stress is probably linked to an increase in blood P4 levels. Saqib et al. (2021) discovered a negative correlation between cortisol and P4 levels. Therefore, it verifies the different functions of the hypothalamus-pituitary-adrenal and hypothalamus-pituitary-gonadal axes. The findings of Saqib et al. (2021) are consistent with the observations made by Mishra et al. (2007), which showed a gradual decrease in blood cortisol levels as the number of post-partum days increased. During the day of oestrous, the cortisol levels in the peripheral plasma reach their highest point because of stress caused by excessive physical activity and the tension related to the oestrous cycle. Saqib et al. (2021) discovered that dairy buffaloes in the later stages of lactation experience enhanced metabolic function, productivity, and reproductive performance while also experiencing decreased stress levels. It demonstrates their capacity to adjust to metabolic, productive, reproductive, and stress-related requirements following parturition.

Insulin-like Growth Factors

Insulin-like growth factor-I (IGF-I) plays a crucial role in the hormonal alterations in mammary gland output following parturition. The liver synthesizes IGF-I in response to growth hormone (GH) and is crucial in regulating development and lactation (Renaville et al., 2002). Multiple IGF-binding proteins (IGFBP) that efficiently suppress IGF activity (LeRoith et al., 2021). In addition, GH opposes the impact of insulin on the body and triggers the secretion of IGF-I. Antagonizing insulin leads to a nutritional partitioning effect, which promotes lean tissue formation and milk secretion (Lucy, 2008). According to Scaramuzzi et al. (2006), GH, IGF-I, and insulin can improve the function of gonadotropin receptors, hence increasing the effectiveness of gonadotropin action. Every hormone could directly influence the ovary by attaching to certain hormone receptors. Metabolic functions like lactation are linked to reproductive processes through a hormonal pathway involving GH, IGF-I, and insulin.

Challenges and Limitation

All hormones face the challenge of the weather. For example, weather conditions can impact plasma E2 levels (Mondal et al., 2006). When comparing E2 levels across different seasons, it was shown that E2 levels were lower during the summer months and the cooler months (Rao & Pandey, 1983). They determined that the main reason for a higher occurrence of silent oestrous during the summer is the decreased peak levels of E2 during oestrous, along with decreasing concentrations of P4.

Furthermore, variables like limited energy intake, decreased body reserve, and post-partum disorders might hinder the process of uterine involution and subsequently delay the restoration of cyclicity. Hence, parturition and the subsequent period are crucial in resuming reproductive function and returning to the normal reproductive cycle (Dhami et al., 2019). An uncomplicated calving process increases the likelihood of a quick return to normal ovarian activity after giving birth. Besides that, it is preferable to have a short NEB. Fertility can be influenced by various factors related to nutrition, management, and the environment (Parmar et al., 2012). The transitional period and early post-partum phase impose biochemical and physiological stress.

HORMONAL CHANGES IN POST-PARTUM DAIRY BUFFALOES

Changes in Progesterone and Oestradiol

In dairy buffaloes, ovarian hormones like P4 and E2 play an important role in determining the oestrous cycle. During the post-partum period, dairy buffaloes undergo substantial physiological and hormonal changes. In the early post-partum stage, there is typically an NEB produced by metabolic stress due to a decrease in the amount of dry matter intake (DMI) and an increase in energy needs during calving and the start of lactation (Mishra et al., 2007; Saqib et al., 2021). The P4 and E2 synthesis patterns may often appear antagonistic, but both are needed for oestrous and ovulation in the buffalo ovarian cycle.

The P4 profiles can be categorised into three basic patterns: normal cycle, prolonged luteal function, and delayed ovulation (McCoy et al., 2006). Others have reported that there are six types of different ovulatory activities based on P4 profiles during post-partum which are normal, delayed or anovulation, cessation or cyclicity, prolonged luteal phase, short luteal phase, and irregular profiles (Opsomer et al., 1998). When calving, the levels of P4 and E2 decreased gradually to their lowest levels, indicating full luteolysis, as shown in Figure 1. The P4 levels decline progressively from the time of calving to the post-partum

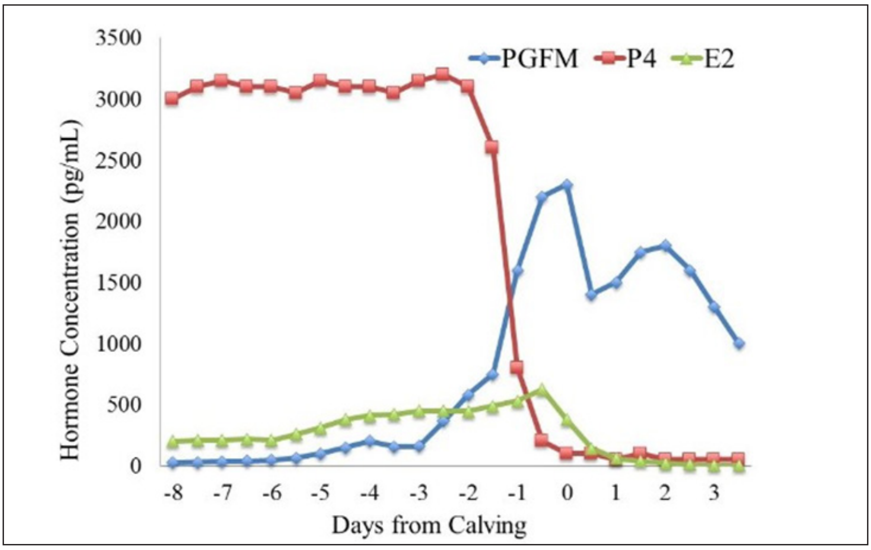

Figure 1. Hormone PGFM, P4 and E2 during the transition period (Mattos et al., 2004; Wiltbank et al., 2016)

period, reaching their minimum point on day 6 (Bahga, 1989) to day 15 (El-Belely et al., 1988). It suggests that the CL of pregnancy has fully regressed. Patel et al. (2020) proposed an extended duration of 3 to 29 days for the complete regression of CL. The decline of CL after calving, as shown by P4 levels on the third day after calving, did not show any variation between milked buffaloes and suckled, according to Arya and Madan (2001). P4 levels remain at a basal level for a varied period of post-partum, but there may be a temporary increase before the resumption of cyclic activity. In late lactation and post-partum stages, buffaloes exhibited improved blood metabolite, P4, and stress levels. The level of P4 rises and falls in sync with the growth and decline of the CL since CL is the source of P4 in cycling bovine (Ahmad et al., 1977).

E2 plays a crucial role in activating the gonadotrophin surge involving the release of GnRH from the hypothalamus and the LH and FSH surge from the anterior pituitary gonadotrophin cells leading to ovulation (Boer et al., 2011). It is well known that E2 is secreted and synthesized by granulosa cells. During post-partum, E2 levels in the blood experienced a considerable decline within the first 24–72 hours. Arya and Madan (2001) stated that basal levels were measured between 2 to 7 days after calving. After this time frame, the levels only had minor fluctuations until day 45 post-partum. Tiwari et al. (1995) showed that the amounts of E2 were higher in buffaloes that were milked compared to those who were suckled. The fluctuations in overall oestrogen levels seen in non-cycling buffaloes during the initial 75 days post-partum are likely indicative of cycles of follicular growth and atresia (Terzano et al., 2012).

Challenges and Limitation

The transition phase, which encompasses the period from late gestation to early post-partum and lasts 6 to 8 weeks, can lead to metabolic disturbances that directly and indirectly impact fertility. Challenging transitions during this period can adversely affect future reproduction (Chapinal et al., 2012). Inadequacies in either the nutritional or non-nutritional aspects of management elevate the likelihood of periparturient metabolic disorders and viral diseases, leading to a decrease in eventual fertility.

P4 and E2 are essential for post-partum dairy buffaloes as they significantly impact oestrous expression and fertility. Inadequate levels can impede the regularity of reproductive cycles and the ability to conceive, affecting reproduction's overall success. Besides that, dairy buffaloes may have P4 insufficiency after parturition during low breeding seasons. It can harm their reproductive efficiency, resulting in silent oestrous and lower conception rates. These effects are evident from the analysis of milk P4 profiles. The reason for this is the insufficient performance of the CL in producing optimal levels of P4. E2 is pivotal in initiating the gonadotrophin surge and ovulation and promoting oestrous behaviour. Consequently, E2 indirectly coordinates the timing of mating and ovulation (Taher &

Hussain, 2022). According to Wathes et al. (2007), the hormonal levels needed for regular oestrous cycles and the return of fertility after calving are gradually returned. Lastly, Failure to recognize oestrous might lead to increased days open, resulting in severe economic losses.

METHODS OF HORMONAL ASSESSMENT

Blood Hormone Assays

Monitoring reproductive cycles and diagnosing the cause of poor reproductive performance in dairy animals is imperative to measuring P4 and E2. There is a need for assays that are easily accessible, can be replicated, provide precise results, and do not involve the use of radiation to measure the levels of P4 and E2 in blood. The dependability and precision of radioimmunoassay (RIA) have made it the preferred method for measuring hormones in dairy buffalo serum, establishing it as the gold standard. RIA presents potential health hazards, has a restricted lifespan, and produces hazardous waste. Using radioactive isotopes in RIA comes with several constraints. Skenandore et al. (2017) propose that ELISA could serve as a feasible alternative. However, it is worth noting that this method may be influenced by various components present in the sample. Sample materials can disrupt enzymeimmunoassay (EIA) methods instead of hormones being tested (Tate & Ward, 2004). Liquid chromatography tandem-mass spectrometry (LC-MS/MS) is the most advanced method for measuring steroid levels in serum. However, it can be costly, time-consuming, and requires specialized expertise, limiting accessibility. Tahir et al. (2013) propose that the chemiluminescence-immunoassay (CLIA) represents a contemporary approach to hormone analysis. Navarro et al. (2022) found that CLIA is more sensitive, faster, and cheaper. P4 levels are commonly measured in human medicine. CLIA's advanced automation enables a high volume of daily analyses, resulting in cost-effectiveness and decreased reliance on human intervention.

Milk Hormone Assays

Milk P4 levels in dairy cows are commonly measured in developed countries to minimize the need for stressful procedures like blood collection, rectal palpation and ultrasound inspection (Wu et al., 2014). Monitoring hormonal changes during bovine pregnancy requires assessing sex hormones in milk (Regal et al., 2012). Measuring P4 in milk presents challenges in sample preservation and varying milk fat content (Comin et al., 2005). However, it offers a simple sampling method. LC-MS/MS is highly regarded for its ability to detect steroids with precision, sensitivity, and accuracy. Electro-spray-ionization linked to tandem mass spectrometry (ESI-MS/MS) is commonly employed for steroid identification in many applications (Regal et al., 2012). Typically, endogenous hormones are non-polar and difficult to ionize using ESI. Derivatization techniques are employed to modify the chemical structure of analytes, facilitating their ionization (Higashi & Shimada, 2004; Regal et al., 2012). Other methods for measuring hormones in milk include ELISA, RIA, and GC-MS, in addition to LC-MS/MS. ELISA is a solid approach technique for testing ovarian cyclicity. This method helps to accurately determine the periods of oestrous and post-breeding, providing the advantages of accuracy and ease of use. A study by Jouan et al. (2006) discovered that using RIA acquired the most dependable data. As previously said, RIA is considered the benchmark. However, it poses risks to human health. Gas chromatography-mass spectrometry (GC-MS) methods offer superior sensitivity and selectivity compared to immunoassays. Nevertheless, these methods usually need laborious derivatization procedures. Thus, Santen et al. (2007) regarded the combination of GC-MS and LC-MS/MS as the most precise methodology.

Applications of Hormonal Profiling in Dairy Buffalo Management

Buffalo has historically been criticized for having a poorer reproductive capacity than *Bos taurus* cattle. Their extended generation gap, caused by lengthy calving intervals (Singh et al., 2000), has been considered a significant barrier impeding genetic advancement in this species. The calving interval in buffalo is influenced by reproductive issues on the female side and management practices. The importance of CL, a temporary endocrine structure on the ovary, in regulating animal reproductive cycles has been well established (Diaz et al., 2002). P4, produced by the CL, is a reliable indicator of luteal function in dairy cows. It can be measured in milk or blood. E2 impacts the central nervous system and can influence oestrous behaviour. Measuring milk P4 and E2 levels is a straightforward and efficient method for monitoring oestrous, ovarian dysfunction, embryonic death, and pregnancy, particularly in rural settings (Osman et al., 2010). Monitoring P4, oestrogen, total protein, cholesterol, calcium, and phosphorus levels in hormone profiling helps assess dairy buffaloes' reproductive status and health (Patel et al., 2020). Additionally, it can detect hormone misuse, assist in veterinary control and ensure food safety.

Challenges and Limitation

Limitations exist in hormonal profiling for dairy buffalo management. These limitations include the possibility of misdiagnosis caused by non-functioning or cystic ovaries. Accurate diagnosis may require multiple milk samples (Osman et al., 2010). Sampling, stress effects, hormone fluctuations, and post-partum diseases can complicate the process (El-Belely et al., 1988). Buffaloes' aggressive temperament and size make handling and sampling challenging. Insufficient CL activity can lead to decreased P4 levels, which can impact reproductive performance and the ability to detect silent oestrous. Various factors hinder the widespread adoption of hormone profiling, such as budget constraints, farmers' hesitancy to disclose production levels and high costs.

FACTORS INFLUENCING HORMONAL PROFILES

Various factors were documented during this review. Figure 2 summarizes factors influencing the hormonal profiles in dairy buffaloes. Some additional challenges and limitations were presented.

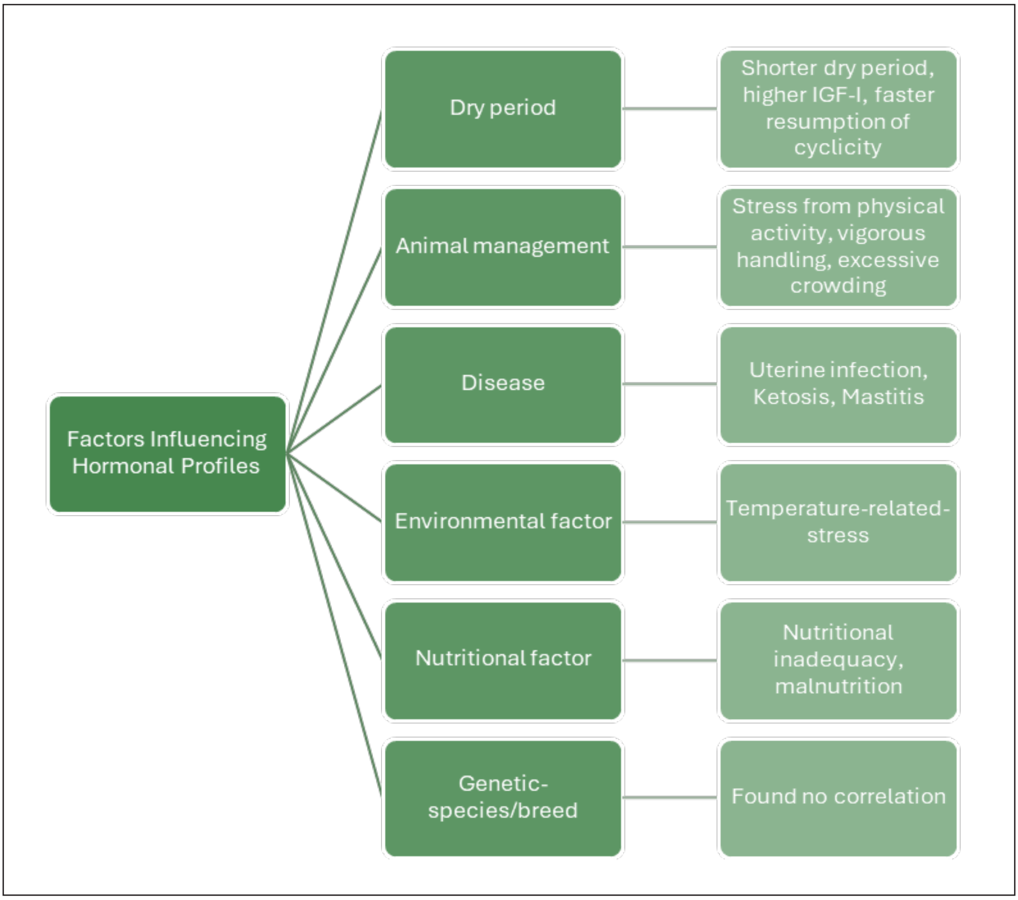

Figure 2. Schematic diagram of factors influencing the hormonal profiles in dairy buffaloes

Dry Period

The dry period is when cows are not milked before calving. Traditionally, this period lasts approximately six to eight weeks (Kok et al., 2019). The dry period serves several tasks. The primary purposes are to administer antibiotics to cows with persistent subclinical mastitis (Bradley & Green, 2001), provide a period of rest for the cow before giving birth to the next calf (Kok et al., 2017), and optimize milk production in the subsequent lactation. A dry period can be regarded as a directly observed economic characteristic of practical importance in dairy farming. The reduction in dry period length lowers milk production after giving birth. However, several researchers observed enhanced milk production in cows either through shortened (Pezeshki et al., 2007) or wholly eliminated dry period (Andersen et al., 2005).

During the dry period, cows exhibited favourable alterations in their hormone profile, characterized by reduced cortisol levels and higher antioxidant status. The alterations led to a rise in milk production during the initial phases of lactation (Bogolyubova et al., 2021). Mollo et al. (2021) stated that the duration of the dry period affects the hormone profile in dairy cows (Figure 2). They found that shorter dry periods are linked to increased levels of insulin-like growth factor-I and a faster resumption of ovarian cyclicity, ultimately improving milk output. A shorter dry period does not affect reproductive performance other than accelerating the return of ovarian cyclicity and improving milk production.

Animal Management

Effective management is crucial for successfully bringing up buffaloes during the summer months. This weakness is further intensified during the hotter season, as seen by Rensis and Scaramuzzi (2003). It is characterized by less apparent signs of oestrus that last a shorter duration. Buffaloes typically display oestrus mainly during the nighttime or early morning hours, sometimes unnoticed by many farmers. Traditional methods of observation are inadequate for identifying oestrus in these animals (Beg & Totey, 1999), leading to longer mating durations in the warmer months. Implementing breeding management approaches such as controlled internal drug release (CIDR) and Ovsynch protocols substantially impacts plasma progesterone levels in buffaloes but does not affect cholesterol and protein profiles (Savalia et al., 2014). Mondal et al. (2010) noted that buffalo management can influence hormone profiles during the oestrous cycle. Factors such as stress from physical activity and oestrous can alter buffalo's cortisol, T3, and T4 levels. Physical activity includes vigorous handling, excessive crowding, and demanding labour.

Diseases

Post-partum infections can affect hormone levels in dairy buffaloes, which can, in turn, affect ovarian function and reproductive efficiency. Ketosis, a serious condition, decreases milk production while lactation (Fiore et al., 2018). Mastitis hampers reproductive success by triggering a systemic immune response and hormonal changes (Risco & Dahl, 2018). According to Wang et al. (2021), it has been found that this phenomenon can cause delays in the reproductive cycle, decrease pregnancy rates, and elevate the risk of abortion. Mastitis can cause damage to follicles, hinder oocyte development, and decrease ovulation capability; it prolongs the time between oestrous cycles, reduces the luteal phase, and impacts embryo development (Edelhoff et al., 2020).

Environmental Factors

Environmental conditions directly influence the neuroendocrine system in buffalo. Buffaloes are highly vulnerable to thermal stress, particularly when exposed to direct sunlight, due to their limited ability to cool themselves through sweat glands, which are present in low numbers. Due to their sparse hair coat, they have minimal protection (Cockrill, 1993). The situation is further intensified by high relative humidity (Misra et al., 1963). Thermal stress induces hyperprolactinemia, decreased frequency of LH, impaired follicle development, and reduced synthesis of oestradiol in anoestrus buffaloes (Heranjal et al., 1979), resulting in ovarian inactivity.

The manifestation and strength of oestrus and heat in buffaloes are diminished during the summer compared to winter, exhibiting a daily pattern (Madan & Prakash, 2007). Increased ambient temperature also reduces the length of oestrus and the manifestation of oestrus symptoms (Upadhyay et al., 2007). The amount of plasma progesterone and its cyclic fluctuations significantly impact oestrus expression, primarily caused by the cyclic changes in the corpus luteum (Bachlaus et al., 1979). The hot months resulted in low levels of progesterone, oestradiol, and luteinizing hormones in buffaloes, reducing oestrus expression and low conception rates (Rao & Pandey, 1982, 1983). The study conducted by Upadhyay et al. (2007) found that during the summer season, the level of follicle-stimulating hormone in buffaloes was lower compared to the breeding season. It suggests that the main reason for the reduced reproductive efficiency of buffaloes during the summer is temperature-related stress.

Nutritional Factors

Typically, dietary factors are commonly associated with post-partum hormonal alterations in bovines. However, diet is merely one of the factors contributing to the seasonal nature of buffalo reproduction (Zicarelli, 1997). According to Cockrill (1993), it is considered a challenge to the development of buffalo. In Egypt, around 36.5% of non-pregnant buffaloes had ovarian inactivity, likely caused by probable nutritional inadequacy (Heranjal et al., 1979). Buffaloes frequently suffer from malnutrition due to the scarcity of nutrients, particularly protein, as tropical forages get lignified throughout the summer months. In addition, elevated temperature also results in a reduction in the amount of food consumed, which is a crucial aspect of the animal's ability to regulate heat (Rahe et al., 1980). The species may experience a decline in body condition and encounter various reproductive limitations due to nutritional restrictions and reduced voluntary feed intake during heat stress.

Genetic-species Breed

Cruz (2007) identifies the Murrah, Mediterranean, Jaffarabadi, Nili-Ravi, Surti, Kundi, Mehsana, Bhadawari and Nagpuri as the leading buffalo breeds in Asia. According to Harun-Or-Rashid et al. (2019), there are no significant differences in oestrogen and P4 levels on day 0 of the oestrous cycle among local, crossbred, Nili-Ravi and Murrah (Figure 3). Earlier, Arora and Pandey (1982) observed that baseline P4 levels during oestrous ranged from 0.1 to 0.3 ng/ml, stabilising around 1 ng/ml for the subsequent 3-4 days. Mondal et al. (2010) found that plasma P4 levels in buffaloes during the oestrous cycle ranged from 0.30 ± 0.06 to 1.94 ± 0.03 ng/ml.

Puberty is initiated by the release of GnRH from the brain, which subsequently increases the secretion of LH. GnRH is vital in regulating LH release, follicular development, and steroid hormone production. Harun-Or-Rashid et al. (2019) observed no significant variations in the duration of the oestrous cycle among local, crossbred Nili-Ravi and Murrah (Figure 3). Mujawar et al. (2019) found that Marathwadi buffaloes have an oestrous cycle duration of 21.25 ± 2.37 days. Various factors, including environmental conditions, nutrition, and abnormalities in ovarian hormones, can affect the duration of the oestrous cycle.

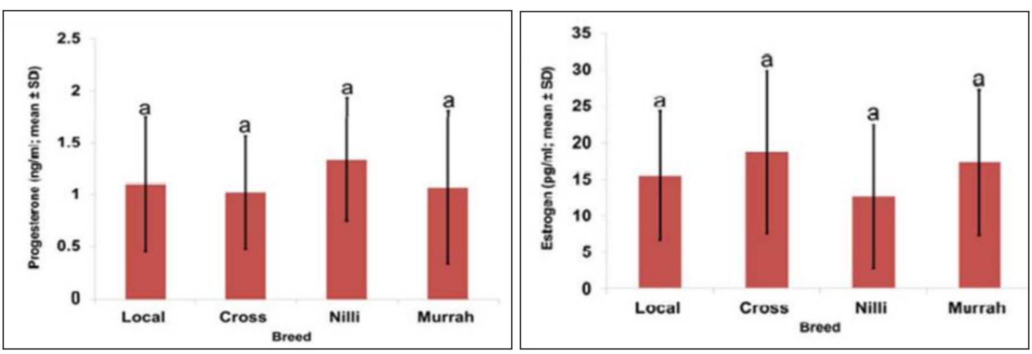

Figure 3. Comparative analysis of ovarian characteristics in several buffalo breeds: (Left) progesterone and (Right) oestrogen levels. Results indicated there is no significant difference ($p > 0.05$) (Harun-Or-Rashid et al., 2019)

Challenges and Limitation

Based on the schematic diagram (Figure 2), buffalo hormonal profiles face difficulties with irregularities in P4, E2, and cortisol levels, as well as metabolic disruptions. Buffalo fertility can be negatively affected by factors, including oxidative stress. In addition, lower LH levels during the oestrous cycle, particularly in warmer months caused by heat stress, impede the initiation of oestrous and reproductive success (Madan & Prakash, 2019). Inconsistent post-partum ovarian activity can result in low P4 levels, leading to conditions like anoestrous, abnormal cycles, and silent heat behaviours (Souza et al., 1997).

In tropical climates, silent heat frequently occurs, which is strongly associated with deficits in management and nutrition, specifically in terms of energy, protein, and minerals (Vale et al., 1988). Furthermore, it is commonly acknowledged that post-partum luteal phases with low progesterone levels might considerably affect the hypothalamic-pituitary-gonadal axis activation (Cable & Grider, 2020). Common irregularities in the oestrous cycle often disrupt fertility. These include shortened or prolonged cycles, silent heat, dysfunctional or persistent CL, inactive ovaries, or ovarian dystrophy. Disturbances like these can hinder heat detection, resulting in lower conception rates and a longer post-partum period. In addition, an NEB during a post-partum period can suppress the quantity and frequency of LH pulses, affecting the stimulation of milk letdown (Souza et al., 1997).

STRATEGIES FOR OVERCOMING CHALLENGES IN HORMONE PROFILING OF DAIRY BUFFALOES

The steroid hormone serves as the principal biomarker indicating the reproductive condition of female animals. Existing methodologies for evaluating the hormone progesterone rely primarily on (enzyme) immunoassays; however, these methods are very expensive for routine screening programs due to their associated labour costs and the necessity for laboratory infrastructure and equipment (Posthuma-Trumpie et al., 2009). It is crucial to know the hormone profiles in dairy buffalo as they have a significant impact on reproductive and production systems. When a dairy animal does not conceive within an optimal timeframe, the farmer faces substantial economic problems, resulting in reduced calf production and milk yield (Bisen et al., 2018). Therefore, it is crucial to enhance the buffaloes' reproductive capabilities managed by small-scale farmers by applying assisted reproductive technologies (Ghuman & Dhami, 2022). Several strategies have been proposed, indicating that hormone profiles in buffaloes can be evaluated by delving deeper into the latest innovations in diagnostic tools and data analytical techniques.

High-performance liquid chromatography (HPLC), gas chromatography, and mass spectrometry represent the analytical methodologies employed to measure progesterone levels in milk (Jang et al., 2017). Applying these methodologies requires extensive sample preparation, a huge financial investment, and skilful personnel adept at operating the laboratory staff to yield the desired outcomes. Limitations such as read-out times, sensitivity, and specificity are inherent drawbacks of lateral flow technology and ELISA kits, which have been used to detect milk progesterone (Wu et al., 2014). Daems et al. (2017) recently introduced a Surface Plasmon Resonance (SPR) biosensor to identify progesterone in dairy milk. This SPR is a competitive inhibition assay with a 0.5 ng/mL detection threshold. Nevertheless, SPR requires a high cost, and it remains a laboratory-centric technique that needs integration with automated milking systems for optimal monitoring (Jang et al., 2017). However, no deployable sensors are available for the on-site assessment of hormone levels in milk.

Enzyme-linked immunosorbent assay (ELISA) is a widely used assay methodology for identifying and quantifying various biological entities due to its remarkable sensitivity and specificity (Song et al., 2023). Frequently, this technique needs the use of substantial and costly laboratory apparatus, which poses challenges for rapid execution at the point-of-care (POC). To enhance portability and user-friendliness, a portable diagnostic system using a Raspberry Pi imaging sensor can rapidly detect progesterone in milk samples, as Jang et al. (2017) outlined. Compared to conventional ELISA assays for progesterone and a commercially accessible plate reader, the proposed POC device demonstrated superior performance metrics while significantly improving portability and simplifying the operation, thus eliminating the requirement for skilful laboratory personnel. The POC reader stands as the sole readily usable portable instrument available to farmers for monitoring the progesterone levels in their herd's milk for various reproductive assessments, effectively addressing a gap with considerable market implications (Jang et al., 2017). Despite all the pros, POC devices need additional validation across a variety of environmental settings to guarantee stable performance within different agricultural systems.

FUTURE DIRECTION

Prospective investigations in hormonal profiling involve integrating endocrinology, cutting-edge agricultural technologies and data science. This amalgamation has the potential to yield a lasting influence on reproduction and productivity across various environmental contexts. Furthermore, subsequent studies are recommended to prioritise advancing diagnostic tools that incorporate artificial intelligence, ensuring they are economically viable and accessible to small-scale farmers, particularly in developing nations such as India and Pakistan, where dairy buffaloes constitute a significant sector of the dairy industry.

CONCLUSION

Ovarian steroid hormones P4 and E2 are pivotal for regulating the oestrous cycle and fertility in dairy buffaloes. Post-partum buffaloes undergo hormonal changes characterized by decreased P4 and E2 levels. These changes are because of reduced DMI and increased energy demands. Normal oestrous cycles and ovulation rely on the appropriate levels of these hormones. Levels of P4 can vary, which can affect the regularity of the menstrual cycle, the duration of luteal function, and the timing of ovulation. E2 triggers the gonadotropin surge needed for ovulation.

Proper nutrition and health management during the transition from late gestation to early post-partum is important to avert metabolic disturbances that may impact fertility. Optimal hormone levels are pivotal for preventing silent oestrous and enhancing conception rates. Monitoring P4 and E2 levels is required in diagnosing reproductive issues. Methods such as RIA can have potential health and environmental risks. ELISA, LC-MS/MS and CLIA are safer and most cost-effective alternatives.

Optimizing milk production and ensuring sufficient rest are key considerations when managing the 6-8 weeks dry period before calving. Managing buffaloes during hot seasons is crucial because their sensitivity to heat impacts the ability to detect oestrous and hormone levels. Mastitis, ketosis, and environmental factors like heat and humidity can disrupt hormonal balance and decrease fertility. Nutritional deficiencies further impair reproductive health during heat stress. It is important to address reproductive challenges through precise hormonal monitoring and improved management practices to enhance reproductive efficiency and productivity in dairy buffaloes. Genetic factors do not directly influence hormone levels.

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