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Parturition and Puerperium in the Buffalo

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As a livestock resource, buffaloes (*Bubalus bubalis*) play an important role in the dairy industry, livelihood generation in rural areas, and rural and national economy, especially in the tropical and subtropical countries [1]. Ability to thrive in hot climates, maintenance and production on poor quality forages, and resistance to tropical diseases are some of the special attributes that make buffaloes more suited to these particular agro-ecological zones [2]. Although buffaloes have some similarities with cattle with regards to various reproductive processes, there are a lot of characteristics that are different and a thorough understanding of these differences is really important for effective reproductive management in the species. This chapter outlines, briefly, the characteristics of parturition and puerperium in buffalo. For a theriogenologist, besides other reproductive processes such as gametogenesis, fertilization, and embryonic development, it is equally important to have a complete knowledge of the basic biology and clinical aspects of parturition and puerperium as these processes have a direct bearing on the overall productive and reproductive efficiency.

1.0. Parturition

Parturition is the process of giving birth. It is triggered by the fetus and involves a complex cascade of endocrine, neural, and mechanical events that promote myometrial contractions, dilation of the cervix, and expulsion of the fetus and the fetal membranes [3-5]. Parturition marks the termination of pregnancy, at which time the fetus is capable of independent existence outside of the uterus [6].

1.1. Signs of Approaching Parturition

The behavioral signs of approaching parturition in buffaloes are similar to those in cattle. Most often, the signs include changes in the pelvic ligaments, enlargement and edema of the vulva, and udder development. Among these, the most obvious and certain indication of approaching parturition is the rapid enlargement of the udder. The changes are useful as a guide and provide an approximate idea of the time of expected parturition but do not permit precise prediction of the exact time of parturition [4,7-10]. One to two days before parturition some buffaloes evidence watery diarrhea which resolves spontaneously usually 24 h subsequent to parturition (Purohit GN, Personal communication). This diarrhea appears to be of psychogenic origin.

1.1.1. Changes in the Mammary Gland

There is a gradual development of mammary gland during pregnancy, however, a rapid enlargement occurs during the last two months of gestation. The enlargement of the udder is more noticeable in primiparous than in pluriparous buffaloes. Udder development becomes more apparent and progressive during the last 7 days of gestation and extremely marked during last 2 to 3 days prior to parturition (Fig. 1). The distension of the teats becomes prominent at about 1.8 days before calving (Fig. 1). The teats become considerably engorged and the superficial mammary veins appear very tense [8,10-12].



Figure 1. Prepartal changes in the mammary gland in buffalo (A: Udder enlargement; B: Teat engorgement). - To view this image in full size go to the IVIS website at www.ivis.org . -

1.1.2. Changes in the Pelvic Ligaments

A week before parturition, buffaloes show sinking of pelvic (sacrosciatic and sacroiliac) ligaments. Just 2 to 3 days before parturition, there is a marked relaxation of the pelvic ligaments except the sacrosciatic ligament that softens about 12 to 24 hours prepartum. Relaxation and sinking of the pelvic ligaments and muscles result in the appearance of an elevated tail head (Fig. 2). The depth of sinking and prominence of pelvis indirectly indicate the degree of relaxation of pelvic ligaments [8,10,12].

1.1.3. Changes in the Perineum and Vulva

The vulva becomes progressively edematous during the last 3 to 4 days and extremely flabby 24 to 36 hours prior to commencement of parturition (Fig. 2). The transverse folds in the vulva disappear completely with the increase in flabbiness of the vulva. Liquefaction of the cervical seal of pregnancy is observed during the last 3 to 4 days that results in a string of clear mucus hanging from the vulva, particularly when the animal lies down [8,10,12,13].



Figure 2. Prepartal changes in the perineum and vulva in buffalo (A: Vulval edema; B: Raised tail-head appearance). - To view this image in full size go to the IVIS website at www.ivis.org . -

1.2. Signs of Labor Pain

As labor pain commences, buffaloes become uncomfortable and nervous, show reduced appetite and less inclination for water, constantly rise and lie down, often look towards their flank (usually right side) (Fig. 3A), hold their head high, and paw the ground. At each contraction, buffaloes raise their tail, arch the back, slightly flex the hocks, and become restless (Fig. 3B). When recumbent, they completely lie down (Fig. 3C) on one side stretching their hind legs [8].



Figure 3A. Behavioral changes during labor in buffalo. Standing with head turned sideways and with an anxious look. - To view this image in full size go to the IVIS website at www.ivis.org . -



Figure 3B. Behavioral changes during labor in buffalo. Restlessness due to pain, the arrow indicates wagging of the tail. - To view this image in full size go to the IVIS website at www.ivis.org . -



Figure 3C. Behavioral changes during labor in buffalo. Sternal recumbency with a nervous look. - To view this image in full size go to the IVIS website at www.ivis.org . -

1.3 Stages of Parturition

Similar to other ruminants, parturition in buffalo is, by convention, divided into three different stages. The first stage is characterized by the dilation of the cervix, the second stage by the expulsion of the fetus, and the third stage by expulsion of the fetal membranes. The duration of these stages and the entire length of parturition, as reported in various studies, are shown below (Table 1).

The first stage involves the dilation of the cervix; complete cervical dilation takes about 0.5 to 2 hours (Table 1). The stage is longer in primiparous than in pluriparous buffaloes. About 12 to 24 hours before parturition, uterine contractions increase in both frequency and amplitude, causing the animal some abdominal discomfort. About 41 minutes (range: 12 to 131 minutes) after the onset of labor and 10 minutes after the termination of first stage, the first water bag (allantochorion) appears (Fig. 4A), progresses forward (Fig. 4B) and ruptures after a mean time interval of 2 minutes. The allantochorion mostly ruptures before it reaches the vulva [3,13,18].



Figure 4A. Sequential events during parturition in buffalo. Arrow indicates appearance of first water bag/allantochorion at the vulva. - To view this image in full size go to the IVIS website at www.ivis.org . -



Figure 4B. Sequential events during parturition in buffalo. Arrow indicates allantochorion hanging from the vulva. - To view this image in full size go to the IVIS website at www.ivis.org . -

Table 1. Duration of Parturition (minutes) in Buffalo (*Bubalus bubalis*)

1st Stage	2nd Stage	3rd Stage	Total	References
70.0±44.30	19.4±15.00	257.0±81.00	302.3	Roy and Luktuke [8]
110.0±10.70	8.9±0.80	271.8±14.10	385.2±18.80	Singh et al. [10]
128.3±14.10	16.6±1.30	544.0±0.02	-	Samadhia [14]
57.5±6.27	14.0±1.01	443.8±24.86	-	Pandey et al. [15]
45.1±2.26	25.5±2.17	325.9±13.87	395.6±14.45	Rawal and Singh [16]
31.6±3.07	43.6±5.25	429.3±43.84	504.5±45.97	Modi et al. [17]
35.2±1.08	43.3±1.21	431.0±46.52	509.4±45.75	Dodamani et al. [18]

After rupture of the first water bag, there is a temporary cessation of abdominal straining followed by the appearance of the fetus contained within the amnion at the vulva. Legs are visible at the external genitalia (Fig. 4C) about 13 minutes on an average after the allantochorion or placental fluid is seen. The dam lies down in sternal or lateral recumbency or alternates between standing and recumbent positions. Strong abdominal contractions and movements of the fetus lead to the rupture of the amniotic sac, and the delivery of the fetus (Fig. 4D), usually in anterior longitudinal presentation, dorsosacral position, with the head resting on the extended forelimbs; posterior presentation is uncommon. Expulsion of the fetal head requires maximum expulsive efforts (Fig. 4D and Fig. 4E). Once the head and limbs of the fetus are out of the vulvar lips the rest of the fetus is delivered easily (Fig. 4F). This stage of labor lasts about 9 to 44 minutes (Table 1), but may extend up to 6 hours, particularly in primipara.



Figure 4C. Sequential events during parturition in buffalo. Arrows indicate second water bag/amnion and fetal limbs appearing at the vulva. - To view this image in full size go to the IVIS website at www.ivis.org . -



Figure 4D. Sequential events during parturition in buffalo. Arrow indicates the appearance of the fetal head at the vulva; notice the marked arching of the back and tight perineum due to extensive straining during expulsion of fetal head. - To view this image in full size go to the IVIS website at www.ivis.org . -



Figure 4E. Sequential events during parturition in buffalo. Fetal head completely out of the vulva. - To view this image in full size go to the IVIS website at www.ivis.org . -



Figure 4F. Sequential events during parturition in buffalo. Fetal hindlimbs coming out of the vulva. - To view this image in full size go to the IVIS website at www.ivis.org . -

As in the cow, the umbilical cord ruptures spontaneously before the calf reaches the ground. As a part of their maternal instinct, buffaloes lick the newborn calves (Fig. 5A, Fig. 5B and Fig. 5C) within minutes of their delivery [3,13,18].



Figure 5A. Feto-maternal interaction after parturition. Arrows indicate the buffalo sniffing the fetus and the glistening, wet hair coat of the fetus. - To view this image in full size go to the IVIS website at www.ivis.org . -



Figure 5B. Feto-maternal interaction after parturition. Arrow indicates the buffalo licking the wet hair coat of the fetus. - To view this image in full size go to the IVIS website at www.ivis.org . -



Figure 5C. Feto-maternal interaction after parturition. Arrows indicate suckling and relatively dry hair coat of the fetus. - To view this image in full size go to the IVIS website at www.ivis.org . -

After delivery, abdominal straining ceases [13] and the fetal membranes are expelled (Fig. 6A, Fig. 6B and Fig. 6C) within about 4.3 to 9.1 hours (Table 1). The expulsion of placenta can be taken as a measure of reproductive efficiency in buffaloes. Heifers maturing early expel their placenta earlier (429 minutes versus 454 minutes) compared to late maturing heifers [19].

A study on the distribution of the population about the time of parturition showed that about 46.7%, 30%, and 23.3% of buffaloes calved in the morning, afternoon, and night, respectively [8]. However, another study reported that the majority of buffaloes calve during day hours [16] while a recent study indicated that 80% of the calvings in buffaloes occur during night hours [18].



Figure 6A. Expulsion of fetal membranes and discharge of lochia in buffalo. Arrows indicate fetal membranes hanging from the vulva. - To view this image in full size go to the IVIS website at www.ivis.org . -



Figure 6B. Expulsion of fetal membranes and discharge of lochia in buffalo. Arrows indicate fetal membranes hanging from the vulva. - To view this image in full size go to the IVIS website at www.ivis.org . -



Figure 6C. Expulsion of fetal membranes and discharge of lochia in buffalo. Arrow indicates sanguineous lochial discharge immediately after parturition. - To view this image in full size go to the IVIS website at www.ivis.org . -

The durations of the different stages of parturition as well as the entire length of parturition have been shown to be influenced by the intensity of labor. Buffaloes that show intense labor pain take lesser time for each stage as well as the entire process of parturition [8,10]. Gender of the calf influences the total time required for parturition; male calf requiring less (305.8±89.5 minutes) time than the female (364.2±108.2 minutes) counterpart [8]. However, parity has no influence on the duration of different stages of parturition [15].

In Murrah buffaloes, the average total number of cotyledons in fetal membranes varies between 117.8±30.3 (range: 67 to 203) to 131.4±11.01 (range: 79 to 195). Their number in the gravid uterine horn ranges from 62.7 to 75.0 and 54.7 to 56.4 in the non-gravid horn. The cotyledons in the non-gravid portion of fetal membranes are smaller in diameter and less in number. Large cotyledons in the gravid horn are mostly present in the middle portion of fetal membranes [8,10,12]. In Egyptian buffaloes, the total number of cotyledons is reported to vary from 42 to147 [20].

1.4. Peripartum Endocrine Changes

Peripartal endocrine changes in buffaloes are basically similar to those described for cattle with the exception that a marked gradual prepartum decline in progesterone is absent. Progesterone is essential for maintaining pregnancy in the buffalo and the maturational events leading to parturition are linked to both the luteolytic effect of corticosteroids and the estrogen-stimulated increased synthesis and release of PGF2α. Plasma concentrations of progesterone remain elevated throughout gestation, however, about 15 days before parturition, plasma levels of both estrone and PGFM increase and reach peak values 3 to 5 days prepartum [21-23]. At parturition, the sharp decline in plasma concentrations of progesterone is associated with a significant increase in plasma concentrations of cortisol [24]; whether the cortisol originates from the mother or fetus, or both, has not been established. Plasma profiles of progesterone around the time of calving revealed a rapid fall in progesterone levels during the last 3 days of gestation [25]. Although plasma progesterone levels decrease markedly on the day of parturition, the estrone and prostaglandin levels decline gradually to basal levels 7 to 14 days after calving [22-23]. A marked difference in testosterone concentrations between pregnant and non-pregnant buffaloes indicates that the fetoplacental unit is active in the production of this steroid; however, sex of fetus has no influence on the testosterone production [26]. Luteinizing hormone (LH) levels started decreasing slowly from Days 261 to 263 and were lowest on the day of parturition but increased by day 16 postpartum [22]. A gradual increase in prolactin concentrations was observed over the last 7 days of pregnancy reaching a peak (2-fold increase) 1 day prepartum [23]. In a recent study on Murrah buffaloes [27], increased interleukin-8 levels were recorded during parturition and postpartum period compared to the prepartum period. This indicates that the peripartal period in the buffalo is associated with an inflammatory response.

2.0. Puerperium

Puerperium is the period after parturition during which the reproductive tract returns to its non-pregnant state in terms of size, location, and function, so that the animal may become pregnant again. Puerperium begins immediately after parturition and lasts until reproductive function is restored [4,28]. Similar to other polyestrous species, a shorter puerperium is desirable for efficient reproduction in buffalo. Involution of the uterus and resumption of cyclic ovarian activity are the two main events that take place during the puerperium.

2.1. Involution of the Uterus

The restoration of the uterus to its normal non-pregnant size and function after parturition is termed uterine involution [3]. Involution of the uterus includes the return of the organ to the pelvic area, to its non-pregnant size with the recovery of normal uterine tone [4]. In the buffalo, uterine regression is considered complete when the uterus returns to its normal non-gravid size and location in the pelvis [29] or when both the uterine horns are approximately equal in size at the external bifurcation and have normal tone [30,31]. Ultrasonographically, uterine involution is deemed complete when both the horns and cervix are visualized as round, echogenic, content-free structures on cross-sectional imaging [32].

A prolonged interval in uterine involution may cause delay in reestablishment of pregnancy and thus result in increased inter-calving period [29]. There is a wide variation in the data on involution time in buffaloes with mean values ranging from 20.1 to 41.6 days (Table 2).

Table 2. Interval from Calving to Uterine Involution (days) in Buffalo (*Bubalus bubalis*)

Breed	Involution Time		References
	Mean	Overall Range	
Murrah (India)	20.1	20.1–39.3	Sane and Desai [33]
	21.1		Sane and Desai [33]
	39.3		Roy and Luktuke [34]
	29.8		Bhalla et al. [35]
Surti (India)	29.0	24.8–31.1	Janakiraman [36]
	24.8		Shah [37]
	31.1		Khasatiya et al. [38]
Nili-Ravi (Pakistan)	25.6	25.6–34.3	Usmani and Lewis [39]
	27.5		Chaudhry et al. [40]
	28.4		Chaudhry et al. [41]
	34.3		Qureshi and Ahmad [42]
Egyptian and Iraqi	35.0	33.3–41.6	El-Wishy [43]
	40.7		El-Sheikh and Mohamed [44]
	33.3		El-Wishy [45]
	41.6		Ali et al. [46]

Part of this variation is because of different approaches used in different studies (transrectal palpation, slaughterhouse based investigation, and ultrasonography), differences in the definition of the initiation and termination time points between various workers, different breeds of buffaloes, and differences in climatic and management conditions [41,47]. The rate of involution of the gravid horn is greater (almost 3 times) than that of the non-gravid horn [34,48,49]. But, the involution of the non-gravid horn is completed earlier than that of the gravid horn [29], owing to a greater difference in size between the gravid and non-gravid horns at parturition. In Murrah buffaloes, the non-gravid horn involuted 11.4 days earlier than the gravid horn in 88.5% of the cases [34].

During the first 24 to 48 hours after calving, the gravid uterus is a large flabby sac lying in the abdominal cavity. At Day 7, the uterus remains cranial and ventral to the pelvic brim. The rate of involution is rapid up to 14 days postpartum and marked decrease in size is observed between 7 and 15 days postpartum. By Day 15 postpartum, the gravid horn can be located at the pelvic brim whereas, the non-gravid horn is in the pelvic cavity but its location is variable [48]. The involution rate gradually decreases and from 21 to 25 days the rate of receding is considerably slow but progressive [34,48]. The complete uterus returns to the pelvic cavity by Day 25 and the gravid horn attains normal tone and consistency by 30 to 35 days [48]. Based on the ultrasonic criteria of visualization of both the horns and the cervix as rounded, echogenic, content-free structures, uterine involution was completed by 25 days postpartum in Bulgarian Murrah buffaloes. Transrectal examination showed that 65%, 82%, and 100% of the buffaloes had their uteri in the pelvic cavity on Days 22, 28, and 34 postpartum, respectively [32]. Histological studies revealed that normal uterine epithelium was reestablished by 30 days on the non-gravid side and 45 days on the gravid side [50].

2.2. Lochial Discharge

Lochia is the uterine discharge that occurs after parturition and contains mucus, blood, shreds of fetal membranes, and caruncular tissues. Approximately 500 ml of lochia are discharged on the first day and decreases gradually [51]. Lochial discharge has been seen for a mean of 6 days range: 0-34 days [42]. The lochial discharge in buffaloes is brown to yellow in color [34]. The color and consistency of lochial discharge changes from day to day. During the first 24 hours, the discharge contains pure blood but on Day 2 and 3, the discharge is more like chocolate color and appears turbid due to a mixture of leukocytes. On the 4th and 5th day, the color changes from chocolate brown to dirty amber, and later becomes light grey tinged with occasional streaks of blood changing to whitish yellow color and albuminous consistency. It gradually decreases in volume and changes to a white mucoid discharge. In the normal course, the discharge usually ceases within 2 to 3 weeks and very negligible thick brownish mucus is passed after the end of third week. After 3 weeks, the discharge becomes very scant [33,51].

2.3. Factors Affecting Postpartum Uterine Involution

Following parturition, the reproductive organs of buffalo undergo involuntary changes to reach their normal size and location. Delayed uterine involution and resumption of estrous cycle prolongs the inter-calving interval and decrease the reproductive efficiency in the species. Various factors like breed and age of the animal, parity, suckling, lactation number, month, season, year of calving and level of production have been reported to influence the postpartum involution in buffaloes. Besides, gestation period, gravid horn, and sex of calf influence the interval to complete the involution of the uterus [34,41,44,47].

2.3.1. Breed

The period of uterine involution may vary with the breeds of buffaloes reared in various geographical locations [41]. The interval between calving to involution of the uterus is reported to vary between 20.1 to 39.3 days in Murrah [33,34], 29.0 to 30.2 days in Surti [36,52], 25.6 to 39.0 days in Nili-Ravi [39,40] and 33.3 to 41.6 days in Egyptian [45,46] buffaloes. However, the involution of the uterus in Kundi buffaloes in Pakistan takes longer (52.0 days) [53].

2.3.2. Age and Parity

Age at first calving influenced the involution of the uterus in Nili-Ravi buffaloes [41]. Egyptian buffaloes aged between 4 to 8 years showed a tendency to a shorter involution time (39.4 days versus 42.7 days) than those aged less than 4 or greater than 8 years [44]. However, the interval between calving to complete uterine involution was similar between primiparous and pluriparous Murrah (20.0 versus 21.1 days), Mediterranean Italian (31 versus 33 days) and Egyptian (37.5 versus 38.2 days; 40.8 versus 40.7 days) buffaloes [30,33,44,54,55]. On the contrary, a significant effect of parity on uterine involution time was reported in Murrah [34,48,56] and Surti [57] buffaloes. Age and parity had no influence on the involution time in Nili-Ravi buffaloes [31,39].

2.3.3. Suckling by Calf

Suckling shortened involution time in Nili-Ravi buffaloes [58]. Limited suckling accelerated uterine involution (suckled, 20.1±2.9 versus non-suckled, 27.7±7.0 days) [31]. Involution rate was reported to be faster in suckled Swamp buffaloes than in river buffaloes [51]. However, other studies suggested that suckling has no influence on uterine involution (suckled, 38.8±1.2 versus milked, 36.9±1.0 days) in Egyptian [30] and Indian Surti (suckled, 29.2±1.82 versus non-suckled, 30.2±1.76 days) buffaloes [52].

2.3.4. Season of Calving

A significant effect of season of calving on the interval to uterine involution in buffaloes was noted in some studies [30,48]. The involution of the uterus and cervix was faster in buffaloes that calved during winter (36.7 and 36.5 days) and spring

(32.2 and 35.7 days) than those that calved during summer (39.2 and 38.2 days) and autumn (42.5 and 41.1 days) months [48]. However, other studies reported that season of calving did not affect involution time [34,41,44].

2.3.5. Milk Yield and Number of Lactations

Studies on the time interval to onset of uterine involution in relation to milk yield are relatively few in buffaloes [47]. Level of milk production had no influence on the rate of uterine involution [48,59]. The intervals to uterine and cervical involution following parturition were similar in low (42.2 and 39.6 days), medium (38.6 and 38.2 days), and high (39.6 and 37.1 days) yielder buffaloes. However, the intervals were prolonged with increasing number of lactations [48]. In contrast, other reports indicated that uterine involution takes a shorter period in low producing than high producing buffaloes [60]. Milk yield had no effect on the involution of non-gravid horn. The gravid horn of high producing buffaloes, however, took more time (38.3 days) for involution compared to low producing (33.5 days) buffaloes [61].

2.3.6. Abnormal Parturition and Uterine Infection

Abnormal parturition (dystocia, retention of fetal membranes, prolapse of uterus/cervix, abortion) and uterine infection (metritis, endometritis, and pyometra) are known to prolong the uterine involution time in buffaloes [42,47-49]. Buffaloes with abnormal parturition took more time (44.0±2.14 versus 38.6±0.88 days) to complete involution compared to normally calved buffaloes [48]. In Nili-Ravi buffaloes, dystocia and retention of fetal membranes delayed uterine involution beyond 60 days [42].

Incidence of delayed uterine involution as a result of bacterial infection was reported to vary between 13 to 25% [43,49,62,63]. Organisms like *E. coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Proteus vulgaris*, *Actinomyces pyogenes*, and *Bacillus* species are the common bacterial contaminants that are isolated from the postpartum buffalo uterus. Among them, *Staphylococcus aureus* is reported to be the most common bacterial isolate [49,62-64]. Cervical and uterine involution may be delayed (10 to 14 days longer) by uterine infection. The mean intervals were reported to be 35.8±6.6 days and 46.3±9.7 days in normal and infected buffaloes, respectively [49].

2.4. Resumption of Postpartum Ovarian Cyclicity

Timely resumption of ovarian cyclicity (ovarian rebound) postpartum is essential for optimum reproductive efficiency and productivity. In buffaloes, besides other factors, ovarian cyclicity is affected by season, temperature, and reproductive maturity of the animals [65-68]. Prolonged postpartum acyclicity and anestrus are associated with long intercalving intervals, thereby, resulting in reduced reproductive efficiency. The conditions are seen more often in primiparous and aged buffaloes, especially when the parturient period coincides with increasing daylight hours [67].

The interval to onset of cyclic activity postpartum based on rectal palpation and progesterone profile ranges from 28 to 71 and 24 to 55 days, respectively [69]. The mean intervals from calving to first postpartum ovulation and estrus reported in various studies are summarized (Table 3).

Table 3. Postpartum Ovulation and Estrus Intervals (days) in Buffaloes (*Bubalus bubalis*)

Postpartum Ovulation Interval	References	Postpartum Estrus Interval	References
42.0±4.00	El-Fouly et al. [70]	56.4±3.90	Usmani [73]
34.0±2.00	Usmani et al. [49]	52.2±4.12	Chaudhry et al. [41]
43.2±7.26	Lohan et al. [71]	48.0±5.00	Usmani et al. [49]
81.6±10.66	Ramoun et al. [72]	59.2±6.02	Lohan et al. [71]
54.0±5.00	El-Wishy [69]	82.0±9.00	El-Wishy [69]
59.4±4.76	Qureshi and Ahmad [42]	69.0±6.03	Qureshi and Ahmad [42]

However, earlier studies reported postpartum intervals to estrus over 100 days in Nili-Ravi (128.7 days; [65]) and Surti (125.8 days; [74]) buffaloes. About 7.2% of the buffaloes showed estrus on or before Day 30 postpartum, 61.8% showed between 31 and 150 days, and 31.0% after 150 days [65].

After calving, there is a rapid functional luteolysis of the corpus luteum of pregnancy, as indicated by a steep decline in the peripheral progesterone concentration. However, structural luteolysis occurs slowly and is usually completed by Day 30 postpartum [13]. There is minimal follicular development on the day of parturition [75] but resumption of follicular activity occurs between 15 to 30 days postpartum [31,76,77]. The follicular activity is more intense in the ovary contralateral to the one bearing the gravid CL, as evidenced by a higher number of follicles greater than or equal to 3 mm in diameter from calving until the first 30 days of ultrasound monitoring [55]. A dominant follicle of more than 8 mm in diameter is first observed by 21 to 24 days postpartum [31,78]. Follicles greater than 10 mm were first noticed at around 26 days postpartum. The duration of growth of the dominant follicle was longer (9.3±0.48 days) than the duration of its regression (7.1±0.40 days) and the largest diameter attained by the first postpartum ovulatory follicle was 13.0±1.10 mm [79]. The growth rate and largest diameter of the first postpartum ovulatory follicle and the interval between the first and second ovulations postpartum did not differ between primiparous and pluriparous buffaloes [55]. Most often, the first postpartum ovulation occurs in the ovary contralateral to the previously gravid horn. Based on progesterone analysis, short luteal phases of 6 to 13 days, sometimes with low progesterone levels, are noted in 26 to 86% of the first formed corpora lutea or just preceding the first observed estrus after calving [69]. These short cycles are followed by long anestrous and anovulatory periods due to prolongation of the inter-luteal interval to 21 to 22 days instead of the normal period of 6 to 7 days, before establishment of regular cyclicity [58].

2.5. Factors Affecting Postpartum Resumption of Ovarian Cyclicity

The postpartum resumption of the estrous cycle is influenced by breed, geographic location, suckling, parity, season, nutrition, managment practices, and other factors [69,80].

2.5.1. Suckling

Suckling has been shown to delay the postpartum resumption of ovarian cyclicity in both river [30,52,58,81] and swamp [82] buffaloes. However, the effect of suckling is complex and is influenced by interaction with other factors such as nutrition, exposure to bulls, and restraint. Proper nutrition and good management alleviate the negative effects of suckling [83]. Suckled buffaloes continuously exposed to bulls with limited grazing have shorter postpartum acyclic and anestrous periods than milked, stall fed buffaloes exposed to bulls [84]. The interval to first ovulation is significantly longer in free suckled than in restricted suckled swamp buffaloes [85,86]. Limited suckling prolongs the postpartum interval to first estrus [31]. The interval from calving to ovulation and first exhibited estrus is reported to be 55.7±8.9 and 63.0±7.2 days in suckled and 39.5±5.8 and 58.8±7.5 days in weaned Egyptian buffaloes [81].

2.5.2. Parity

The effect of parity on the resumption of cyclic activity postpartum is not clear in buffaloes. While some studies [55,57,87,88] reported an inverse relationship between parity and resumption of ovarian cyclicity, others [30,89] indicated no association. The inconsistency could be due to potential confounding effects of interaction with other factors such as age, lactation, and body weight.

2.5.3. Nutrition

A number of studies in river [58,90] and swamp [85,91] buffaloes showed that prepartum nutrition does not affect postpartum resumption of ovarian activity. However, a few studies [92,93] indicated a beneficial effect of high plane of prepartum nutrition on postpartum ovarian activity.

2.5.4. Season

Several studies have demonstrated the significant influence of season on postpartum ovarian activity in buffaloes [94,95]. However, the mechanism of such effects continues to be poorly understood [80]. The interval to the development of first palpable follicle (>8.0 mm in diameter) averaged 21.4±0.8 days across the seasons [31]. Buffaloes calving during the normal calving season resumed ovarian cyclicity earlier than those calving during the other seasons [13,96,97]. Nili-Ravi buffaloes that calved during autumn (104.7 days) had shorter postpartum estrus intervals than those that calved during other seasons (spring 174.6 days; summer 124.6 days; winter 151.6 days) [65]. Seasonal influence on postpartum estrus interval was also recorded in Egyptian buffaloes but with a different trend; buffaloes that calved during autumn showed the longest (163.0 days) interval to postpartum estrus followed by winter (153.0 days), spring (145.0 days) and summer (79.4 days) [44]. Furthermore, the month of calving had a significant effect on postpartum estrus interval interval of Nili Ravi buffaloes; it was shortest in buffaloes that calved during the month of September (84.4 days) and longest in buffaloes that calved during March (178.0 days) [65]. In India the shortest and longest intervals were recorded in October (63.0 days) and February (229.3 days) respectively [74]. However, some studies [30,87,98] reported that season of calving did not affect the interval to resumption of ovarian cyclic activity postpartum.

2.5.6. Other Factors

Uterine infection does not affect the interval to first postpartum ovulation and life span of the corpus luteum formed after the ovulation but prolongs the interval to first postpartum estrus [49]. A high proportion (28 to 68%) of buffaloes undergo ovulation without estrous behavior during the early postpartum period [49,71,99]. The fertility rate for the first 120 days postpartum and the number of services per conception were not affected by infection but the mean service period was significantly longer in buffaloes with a uterine infection [49].

2.6. Postpartum Endocrine Changes

Information on hormonal changes that occur during the postpartum period is meager in buffalo. Available evidence suggests that there are changes in levels of several hormones, notably gonadotropins, ovarian steroids and PGF2α, during the puerperal period.

The basal concentrations of follicle stimulating hormone (FSH) remain very low during the early postpartum period [100,101]. On Day 2 postpartum, the concentration was 7.0±0.8 ng/mL. The concentration increased with time and reached 11.8±1.7 ng/mL on Day 20 [100,102]. This increase in the concentration of FSH around Day 20 is thought to be associated with the emergence of new follicular wave that results in the development of large follicles (8 mm) for the first time at 24.4±2.0 days postpartum [79]. The concentrations increased further and on Day 35 reached 12.0±1.8 ng/mL [100,102]. Suckling did not affect the FSH levels postpartum [101].

Luteinizing hormone (LH) concentrations remain low throughout the puerperal period [23]. The values ranged from 0.2 to 0.8 ng/mL during the first 21 days postpartum. In the early postpartum period (second and third week), basal plasma LH concentration is inversely related to the value on the day before first postpartum ovulation [103]. The concentration of LH increased by Day 16 postpartum [22] and at around Days 20 and 35 the mean values were 1.3±0.0 and 2.2±0.1 ng/mL, respectively [100]. In postpartum buffaloes with no history of estrus or ovulation, low serum LH concentrations were noted during the first 120 days (0.6 to 1.1 ng/mL) with no significant temporal differences [104]. Basal LH levels were not different between suckled and weaned buffaloes during the postpartum period [101].

Estradiol-17β concentration in plasma fluctuates widely during the postpartum period. In Murrah buffaloes, there is a steep decline over the first 3 days following parturition [22,105,106] followed by a gradual decline on Day 5 [105]. Basal values were recorded between Days 2 and 7 after parturition [107-109] with minor fluctuations between 10 to 18 pg/mL [105,110] until estrus, when it reached about 20 pg/mL [105], or on Day 45 postpartum [110]. In Surti buffaloes, estradiol-17β concentration fluctuated between 23.3±4.0 to 28.9±3.1 pg/mL during the first week postpartum. The relatively higher value of estradiol during the first week postpartum is suggested as being indicative of resumption of ovarian activity and early onset of postpartum estrus [111].

Following a precipitous fall around the time of parturition [47], plasma progesterone levels decrease linearly [105] and remain basal for a variable period postpartum [112-114]. Progesterone levels varied between 0.4±0.1 to 0.7±0.2 ng/ mL during Days 1 to 7 postpartum in Surti buffaloes [111]. In Murrah buffaloes, the values were 0.6±0.1 ng/mL on Day 7 and 0.5±0.2 ng/mL on day 15. The levels remained low (0.4±0.1 ng/mL) until Day 30 but increased to 1.3±0.4 ng/mL on Day 45 postpartum [115]. The continued decline in the concentration of progesterone until it reaches minimum levels between Days 6 to 15 was also reported in later studies [25,116]. However, in some buffaloes, basal values are attained around Day 29 postpartum [105]. Besides, a transient rise of progesterone levels was reported prior to the resumption of cyclic ovarian activity [112-114,117], which is probably necessary for priming the neural centers for manifestation of estrus behavior postpartum [118].

Temporal changes in the peripheral concentrations of the metabolite of PGF2α, commonly referred to as PGFM, have been reported peripartum in the buffalo. The concentrations increased gradually during the last 7 to 15 days prepartum [21,23,108], peaked at around the time of parturition, and decreased afterwards. The concentrations declined from 4.0±0.3 ng/mL at parturition to 0.4 to1.3 ng/mL on Day 3 [110,119]. Similarly, a decrease from 14.0±2 ng/mL to 5-8 ng/mL on Day 6 was reported in Egyptian buffaloes [108]. The concentrations decreased further, ranging from 0.1 to 0.2 ng/mL, and reached basal values at 10 to 20 days postpartum [21,120,121].

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