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Fertility Augmentation Approaches in Dairy Animals - A Review

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A B S T R A C T

Fertility is one of the key determinants of the life time reproductive performance of the dairy cattle (*Bos taurus and Bos indicus*) and riverine buffaloes (*Bubalus bubalis*) which is reflected by its genotype, physiological condition, nutrition and management. It is assessed by the ability of a dairy cow to produce one calf per year. Fertility in riverine buffalo is considerably lower than in cattle. To attain the optimum reproductive performance the animal has to have functional ovaries, display estrous behaviour, mate, conceive, sustain the embryo through gestation, parturiate and resume normal reproductive cycle, but any deviation from this physiological phenomenon which limits the reproductive performance of the animal is known as infertility. Infertility in the dairy herd leads to a loss of milk production, a loss of income from calf sales and an increase in the replacement rate of the cows with first calving heifers. Accurate estrus detection is considered as key determinant for efficient reproductive management of the dairy farm. The following factors have much negative impact on fertility and therefore need to be plan out to ascertain with such conditions. Firstly, reduce the negative energy balance and resolve any kind of infection from the reproductive. Secondly, the estrus detection efficacy and efficiency should be elevated with proper management and use of novel estrus detection techniques. Accurate and timed estrus detection should be followed by artificial insemination (AI) with elite germplasm by a qualified technician. Further, adoption of other novel assisted reproductive techniques/regimes like timed insemination protocol, superovulation, synchronization of estrus; embryo transfer technology (ETT) etc. in dairy animals is demanded for the benefit of the livestock owners.

Introduction

It has been long recognized that, although a cow that appears to have an apparently structurally and functionally normal reproductive system is inseminated or served at the correct time, with fertile semen, may fail to become pregnant. Regular monitoring of the dairy herd in view of fertility management is the key to determine the reproductive efficiency of the dairy herd which is a single index that provides an overall measurement of the fertility and takes into account many different parameters. Reproductive efficiency is high priority in all systems yet is considered higher in seasonal calving systems as the opportunity for a cow to calve and become pregnant is time limited to ensure a calf per cow per year (Dillon *et al.,* 2006). Consequently, one of the leading challenges before theriogenologists, geneticists and nutritionists is to gain the understanding of the underlying etiologies which contributes towards low fertility and also to develop strategies to improve the conception rate. Fertility is a multi-factorial trait and its deterioration has been caused by a network of genetic, environmental and managerial factors and their complex interactions make it difficult to determine the exact reason for this decline. However, the congenital abnormalities are much less common than those which are acquired. Kessy (1978), in 2000 genitalia collected from abattoirs, observed that only 6 specimens (0.3%) had evidence of congenital abnormalities compared with 194 (9.65%) with acquired lesions. Buffaloes are considered difficult breeder because of their inherent susceptibility to environmental stress (summer or heat stress) which cause anestrus and repeat breeding, besides delayed age at first calving and higher incidence of silent ovulations (Gokuldas *et al.,* 2010). These two conditions i.e anestrus and silent estrus are mainly responsible for a prolonged inter-calving period resulting in great economic losses for the buffalo dairy industry in countries like India, consisting more than half of the world's buffalo population. An inadequate knowledge about buffalo reproduction continues to hamper our progress towards countering the reproductive problems limiting its productivity (Das and Khan, 2010). Despite this, researchers have identified many key causes occurring during the productive life of the dairy cow that hampers their reproductive efficiency. Hence, poor reproductive efficiency in lactating dairy animals limits the economic viability of commercial dairy operations.

Reproductive efficiency is the key determinant influencing productivity of the livestock and is adversely affected by late attainment of puberty, silent estrus, variability of estrous length, seasonality of calving, dystocia, genital prolapse, and retention of fetal membranes, long postpartum anestrous and subsequent calving interval (Barile, 2005). Infertility among dairy animals continues to be major bottleneck in exploiting the fullest production potential of our animal wealth. An annual loss due to reproductive inefficiency is more than 500 crore rupees (Dairy India, 2007). Low reproductive efficiency in the buffalo remains a major economic problem globally and its incidence is higher in India (Kumar *et al.*, 2009). Prolonged postpartum acyclicity and anestrus are major causes of economic loss to buffalo breeders (El-Wishy, 2007). High reproductive efficiency is necessary for efficient milk production and it, therefore, has an important influence on herd profitability (Pryce *et al.,* 2004). Low reproductive efficiency decreases herd profitability by: (i) prolonging the calving interval, which results in less milk production per cow and fewer calves born per year; (ii) increasing culling rate due to infertility and therefore, increasing replacement costs; (iii) increases labour cost, insemination cost and veterinary health service charges; (iv) an extended low production or dry period can result in over conditioned cows calving in too high a body condition score (BCS) (>3.5) which results in a subsequent prolonged period of negative energy balance and low reproductive efficiency. The two key components of high reproductive efficiency are: High submission rate of cows for insemination and secondly, high conception rate to each service. Submission rate is defined as the percent eligible cows (>45 days post-partum) presented for artificial insemination (AI) in a 24-day period (representing an estrous cycle). The two key factors that affect it are percent dairy cows that are anestrus and the estrus detection efficiency and efficacy in the herd. The target is to achieve >80% of cows inseminated in a 24-day period of the breeding season. Therefore, to obtain high submission rate to AI, it is necessary to obtain proper uterine involution by day 50 and get >90% of cows resuming cyclicity with normal estrous cycles before breeding (Roche, 2006).

Genetic makeup and managerial practices

In recent years, the emphasis within selection indices for Holstein-Friesians has shifted predominantly from production to functional non-production traits associated with improved health and fertility (Miglior *et al.*, 2005). It has been established that the phenotypic historical decline in fertility has reached a nadir and begun to improve (Norman *et al.*, 2009); however, future prospective studies are demand of the time to confirm this trend and to determine the alleviating factors. Other reasons for the decline in fertility including poor nutrition, management practices and environmental factors are often not evaluated in detail although they have a significant impact on reproductive performance. Hence, inappropriate management of high milk producing dairy cows may significantly contribute to the cause of poor fertility rather than direct genetic effects (LeBlanc, 2010).

Progeny testing of buffalo bulls based on the milk production of its offspring is under progress since 1993 in India under 'Network project on buffalo improvement.' Field progeny testing for improvement of different breeds of buffaloes at different centers started in 2001. Unlike lactating dairy cows, nulliparous dairy heifers with similar genetic merit for milk production have higher conception rates (39% vs. 64%, respectively) and this has not decreased during the period of genetic improvement in milk yield (Pryce *et al.*, 2004). Hence, it is reasonable to suggest that demands of high milk production negatively impact a number of physiological pathways to reduce the likelihood of the concomitant establishment of pregnancy and those changes in management practices may go a long way to provide solutions to poor fertility in high producing animals. Therefore, it is important that the proper health and nutritional management strategies are optimized in parallel with a more balanced breeding program in order to produce a more robust dairy cow capable of both high productive and reproductive performance.

Factors affecting subsequent fertility in the early post-partum period

The reproductive and other physiological events occurring in the post-partum period of the dairy cattle and buffaloes are significantly affected by the care and management of the dam before and during the calving process. Poor nutrition during the pre-partum period can predispose the animal to metabolic disorders at calving with inability to give birth to the young one with resultant dystocia and negative energy balance. At the other extreme, high dry matter intake increases the metabolic clearance rate of steroid hormones and this can lead to sub-estrus/silent estrus, poor estrus detection, anovulation, reproductive tract disorders like endometritis, pyometra etc. ultimately leading to increased calving to conception period. The reproductive and metabolic disorders occurring due to negative energy balance and deficiency of minerals like calcium, magnesium, phosphorus lead to production and economic losses to dairy farmers. It is becoming increasingly evident that fertility is declining with rising milk yields shifting the criteria of selection from production to non-productive traits having impact on improved fertility and health of dairy animals. However, there is no clear consensus regarding the mechanism of the effect of milk yield on fertility and subsequent reproductive performance. These effects are elaborated below in detail.

Body Condition Score (BCS) loss and negative energy balance

High productive dairy animals require a considerable increase in energy requirements to facilitate the dramatic increases in daily milk yield, which peaks between 4 and 8 weeks postpartum. This requirement is partially met by increased feed intake with the remainder being met by mobilization of body reserves which predisposes the animal to negative energy balance. The consequences of severe NEB are metabolic diseases like ketosis, reduced immunity and impaired fertility (Roche *et al.,* 2009). Body condition score is an internationally accepted, subjective visual and tactile measureable parameter of body condition and temporal changes in BCS are used to monitor nutritional and health status of high producing animals during their productive cycle which has direct as well as indirect effect on the reproductive performance of the animal (Berry *et al.,* 2007). It has been correlated with reproductive performance, both phenotypically (Buckley *et al.,* 2003) and genetically (Berry *et al.,* 2003) and supports the premise that nutritional status affects reproductive function considerably.

Animals in low BCS at parturition, or that suffer excess BCS loss in early post-partum period, are less likely to have poor conception rate or increased early pregnancy losses. Therefore, increased calving to conception interval decreases the reproductive efficiency in farm animals. Animals that are over conditioned at calving (BCS≥3.5; 5-point scale) are also compromised as they have reduced dry matter intake (DMI) just prior to parturition, take longer to increase DMI post-partum, tend to have greater fat mobilization and therefore a more severe negative energy balance associated with metabolic disorders like ketosis (Roche *et al.,* 2009). Environmental stress like summer or cold stress can further aggravate the effects of NEB. During periods of heat stress, lactating cows have a reduced appetite and increase in BCS loss in the early postpartum period compared to non-heat stressed animals. Furthermore, concentrations of glucose, IGF-I and cholesterol are lower, while concentrations of non-esterified fatty acids (NEFA) and urea are higher in blood and follicular fluid of heat stressed animals (Shehab-El-Deen *et al.,* 2010).

The ratio of milk fat: milk protein percentage is a useful predictor of dairy animal with a high risk of negative energy balance, ketosis, displaced abomasum, ovarian cyst, lameness, mastitis and higher likelihood of body condition score loss of greater than 0.5 units (Heuer *et al.,* 1999; Hamann and Kr¨omker, 1997; Buckley *et al.,* 2003). Although Heuer *et al.,* (1999) used the milk fat: protein ratio of 1.5 for early lactation cows to indicate problem cows, other studies suggest that a lower value closer to 1.3 should be used (Buckley *et al.,* 2003; Hamann and Kr¨omker, 1997). Heuer *et al.,* (2000) described cut-off points for energy balance in early lactation using milk variables as milk fat: protein ratio > 1.4, milk protein < 2.9%, milk fat > 4.8% and milk lactose < 4.5%.

Deficiency of minerals and vitamins during transition period

Minerals and vitamins play a crucial role in the reproduction. Transition period is the stage of reproductive cycle in the lifetime of dairy cattle when she is more susceptible to reproductive disorders. The peri-parturient diseases like abortion, still birth, pre-mature birth, antepartum cervicovaginal prolapse, uterine torsion, post-partum uterine prolapse, dystocia, retention of fetal membranes (RFM), immunosuppression and metabolic deficiency disorders like hypocalcaemia, hypophosphatemia, hypomagnesaemia hampers the reproductive efficiency of the dairy animals. Calcium deficiency or hypocalcaemia that results in atony of genital organs, could predispose the animal for prolapse of genital organs (Pandit *et al.*, 1982). Selenium deficiency has also been reported to cause prolapse of genitalia (Diminov *et al.*, 1988). Beneficial effects of addition of β-carotene and vitamins A, E and C in sufficient amounts to the rations of the animals before parturition reduce the reproductive problems (Yildiz *et al.*, 2005). Supplementing β-carotene and α-tocopherol in cows during dry period will improve the immune status thereby, reduces the reproductive problems during peri-parturient period. Increased incidence of retained placenta with keratinization and degeneration of the placenta, metritis are observed in animals fed on vitamin A deficient diets (Chawla *et al.*, 2004). Clinical signs of infertility related to vitamin A deficiency include delayed onset of puberty, abortion, birth of weak, blind or in-coordinated calves (Schweigert, 2003). Vitamin D is having specific roles in reproductive tissues via calcium homeostasis (McDowell, 2000). Vitamin E is involved indirectly in prostaglandin synthesis (Hurly *et al.*, 1989). Vitamin E enhances the development of bovine embryos *in vitro* (Reis *et al.*, 2003). Supplementation with vitamin E and Se has reduced incidence of retained placenta and improved reproduction of dairy cows (Thomas *et al.*, 1990). Calcium and glucose can be supplemented during the later part of pregnancy as a prophylactic measure against retained fetal membranes in cattle (Mohanty *et al.*, 1991). The uterine involution period was shorter in buffaloes treated with vitamin E and Se (Quareshi *et al.*, 1997).

Retention of fetal membranes (RFM)

Retention of fetal membranes occur due to any interference with the normal detachment of cotyledons from the caruncle resulting in its failure to be expelled within 12 hours of parturition. The incidence of RFM in a healthy herd of cows on an average has been found to vary from 5-15%. The enzymes involved in tissue remodeling such as collagenases and proteases play an important role in conjunction with myometrial contractions in the early post-partum period. Decreased neutrophil migration, manifest as reduced placental attraction, is implicated as a primary cause of RFM (Kimura *et al.*, 2002). Interleukin-8, a potent chemo-attractant and the immune status were reduced in cows that develop RFM. Milk fever has also been reported to increase the risk of RFM by 2.5 to 4 folds (Olsen, 1993). Increased metabolic stress could be implicated in the earlier but reduced synthesis of cortisol, the reduced estradiol and prostaglandin PGE and F metabolites before parturition in cows with RFM's (Wischral *et al.*, 2001). RFM delay uterine involution, predispose cows to endometritis or metritis and decrease fertility and reproductive performance (Maizon *et al.*, 2004). The nutritional factors involved are: high BCS at calving, nutritional deficiencies of Vitamins A, D and E and deficiencies in selenium, iodine and perhaps zinc (Gupta *et al.*, 2004; Han and Kim, 2005). Hypocalcaemia is also a key risk factor for RFM (Houe *et al.*, 2001). Thus, maintenance of normal uterine physiology by good nutritional management during the dry and transition periods is important to reduce the incidence of RFM, which is an important risk factor for endometritis causing increased calving to conception interval and decreasing the reproductive efficiency of the dairy herd. Eiler and Hopkins (1992) found that placentomes when incubated with collagenase, facilitate placental separation.

Metabolic disorders

During the period ranging from 2 weeks pre-partum to about 4 weeks post-partum

(Transition period), there is increased demand for energy and protein to meet milk production combined with reduced feed intake which is generally inadequate to meet the maintenance and production requirements. Thus, animal enter a period of NEB characterized by marked alterations in endocrinological, metabolic and physiological status of the animal leading to compromised immune system resulting in decreased BCS and milk production. Immunocompromised animals are at risk of developing metabolic and reproductive disorders, which include ketosis, fatty liver disease and retention of fetal membranes. Metabolic disorders such as clinical hypocalcaemia, hypomagnesaemia and ketosis, caused by a mismatch between macro-mineral requirements and availability in the diet such can further exacerbate the degree of immuno-compromisation in early lactation (Mulligan and Doherty, 2008). Animals that experienced metabolic disorders in peri-partum period are more likely to have increased incidence of mastitis, lameness and endometritis all of which ultimately contribute to reduced reproductive efficiency. Thus, adoption of nutritional strategies during the dry period and early post-partum is the principle route to minimizing the effects of NEB, reducing BCS loss and thereby avoiding the development of metabolic disorders (Roche, 2006).

Uterine and udder health

The normal uterus is able to effectively neutralize the bacterial infection after calving via strong uterine defence mechanism which includes non-specific phagocytosis by neutrophils, uterine gland secretion and myometrial contractility. Uterine infection at calving or in the following days is unavoidable and normal with 80–100% of animals having bacteria in the uterine lumen in the first 2 weeks post-partum. The most common uterine pathogenic bacteria in infected animals include *E. coli*, *Streptococcus* spp., *Staphylococcus* spp., *Pseudomonas* spp., *Clostridium* spp., *Pasteurella multocida*, *Arcanobacterium pyogenes*, *Fusobacterium necrophorum*, *Bacteroides* spp., *Prevotella melaninogenica* and *Proteus* spp. Most of the animals successfully deal with this bacterial infections; however, at least 20% of animals are unable to resolve the infection and develop clinical metritis within 21 days post-partum and infection persisting for more than 21 days in approximately 15–20% of the herd, results in clinical endometritis (Sheldon *et al.,* 2009). The most efficacious methods for its diagnosis include the presence of muco-purulent discharge, vaginoscopy, the presence of an enlarged cervix and/or the presence of inflammatory cells in uterine aspirates collected by cytobrush technique.

The prevalence of endometritis varies with method of diagnosis used, production system employed, yield and parity of cows being examined. The risk of uterine infection is increased in animals with twins, premature birth, abortion, stillbirth, uterine torsion, dystocia or retained fetal membranes. Future therapy of bovine endometritis including treatment with immunomodulators like *E. coli* lipopolysaccharide (LPS), oyster glycogen, serum, plasma or hyper immune serum, PMN extracts and components, leukotriene B4 (LTB4), enzymes like lysozymes and collagenase, granulocytes-macrophage colony stimulating factor (GM-CSF) and inmodulen (LPS+*Propionibacterium granulosum*) needs to be validated for effective treatment of reproductive tract disorders.

A positive genetic correlation between mastitis and milk yield has been reported (Ingvartsen *et al.*, 2003) and consequently high yielding animals have an increased risk of developing mastitis. Buffaloes are less susceptible to mastitis as compared to cattle due to their long teat canal.

Table.1 Herd reproductive targets (Noakes *et al.*, 2009)

S	Index	Target
1	Mean calving to first service interval (days)	65
2	Mean calving to conception (pregnancy) interval (days)	85
3	Mean interval from first service to conception (pregnancy) (days)	20
4	First service submission rate (%)	80
5	Overall pregnancy rate (%)	58
6	First service pregnancy rate (%)	60
7	Reproductive efficiency (%)	46
8	Cows served that conceive (%)	95

Table.2 The postpartum reproductive targets to be met to obtain high reproductive efficiency and the associated key risk factors affecting these targets (Roche, 2006)

Reproductive process	Target to be achieved	Risk factors affecting targets
Normal uterine involution	Day 50 post-partum	Dystocia, RFM, Uterine infection
Resumption of ovulation	90% by day 42	Loss of > 0.5 BCS unit Low feed intake Uterine health
High estrous detection	85% per cycle	Infrequent checks Sub-estrus High yield
High conception rate to AI	50% per breeding	Excess BCS loss Prior uterine problems

Animals that experienced clinical mastitis in the first 28 days after calving had delayed onset of estrus behavior (91 days) compared to their healthy herd mates (84 days) (Huszenicza *et al.*, 2005). Cows with clinical mastitis required more services per conception compared to their healthy herd mates (2.1 vs. 1.6, respectively) and had longer days empty (140 vs. 80, respectively) (Ahmadzadeh *et al.*, 2009). Lameness is also associated with increased number of services per conception and consequently lower conception rates to first service (Hernandez *et al.*, 2001). In conclusion, high productive animals have reduced immune competence that can lead to increased incidence of lameness, mastitis and endometritis. For better reproductive efficiency main focus should be on increasing dry matter intake during the transition period, minimizing NEB and decreasing BCS loss during early post-partum period.

Ovarian rebound

A 'normal' post-partum dairy cow can be defined as one which has resolved uterine involution, have cleared the post-partum uterine micro flora, resumed ovarian follicular development, ovulated a healthy dominant follicle early postpartum and continues to have normal estrous cycles with behavioral estrus at regular intervals of approximately 21 days, coupled with homeostatic concentrations of insulin, IGF-I and glucose (Roche, 2006). The predisposing factors for delayed resumption of cyclicity include peri-parturient disorders like dystocia, ante-partum cervico-vaginal prolapse, uterine torsion, post-partum uterine prolapse, retention of fetal membranes and metabolic disorders like milk fever, ketosis, hypomagnesaemic tetany, post parturient haemoglobinuria, season of calving, management, mastitis, lameness and severe BCS loss (Crowe, 2008). Heat stressed animals may also have low LH pulse amplitude and frequency, low estradiol concentrations and smaller dominant follicles which can extend the interval from calving to first ovulation (De Rensis and Scaramuzzi, 2003) and decreases the reproductive efficiency of dairy herd.

The reproductive efficiency in buffalo is so alarmingly low that it poses a very serious threat of economic loss to Indian and animal husbandry professionals. The resumption of cyclicity, postpartum is a critical factor to achieve a satisfactory production in buffaloes. In dairy animals it should be about 60-80 days and conception must be obtained by 85-100 days post-calving to get desirable benefits. Unfortunately, post-partum estrus in buffaloes always comes much later than this figure.

Elongation of post-partum anestrus interval was found in riverine buffaloes those produced more than 8 kg milk per day than those produced less than 8 kg milk per day (El-Azab *et al.,* 1984). The long post-partum anestrus in the buffalo depends on several factors including: season of calving, peri-parturient disorders, uterine pathology, uterine involution, suckling, milk yield, nutrition and body condition score at calving (Nam, 2010).

In dairy cows the anestrus is due to failure of dominant follicle (DF) to ovulate rather than to their absence (Roche *et al.,* 2000). The DF undergoes atresia rather than ovulation due to its failure to produce sufficient concentrations of estradiol to induce a pre-ovulatory gonadotrophin surge and hence, ovulation (Roche and Diskin, 2000). The inability of the DF in anestrus cows to produce elevated concentrations of estradiol is related to the degree of NEB in the early postpartum period (Stagg *et al.,* 1998) which causes the following effects on: Firstly, reproduction (a decrease in LH pulse frequency, diameter of DF, estradiol production, systemic IGF-I and increased interval to first estrus). Secondly, metabolism (higher growth hormone production and uncoupling between growth hormone receptor and IGF-I production. Thirdly, decreased body condition score with diminished glucose and insulin concentrations. Lastly, higher NEFA, beta-hydroxy butyrate and triacyl glycerol concentrations) (Roche *et al.,* 2000).

In conclusion, management factors stimulating the early resumption of cyclicity, lead to a number of estrous cycles before insemination, and ultimately result in more animals getting pregnant than poorly managed dairy animals that have prolonged periods of post-partum anestrus.

Factors affecting fertility during the breeding season

Timely rebreeding with adoption of fixed artificial insemination protocols (FAI) in post-partum lactating cows is essential for reducing average days open and the corresponding calving interval. A successful breeding program enhances profitability by maximizing the time cows spend in the most productive portion of lactation. The rate at which cows become pregnant in a dairy herd, commonly called the pregnancy rate, is defined as the number of eligible cows in a herd that conceive every 21 days. Two major factors determining pregnancy rate are: 1) conception rate or pregnancy rate per artificial insemination, and 2) estrus detection rate or service rate. The factors affecting estrus detection and conception are discussed below:

Estrus behaviour

Although buffaloes are polyestrous, their reproductive efficiency shows wide variation throughout the year. Water buffaloes have a cycle length of 21 days (range 18–24 days), and an average duration of estrus of 18 hours (range 5–36 hours). Compared to cattle, estrus behavior in water buffalo is much subtler, and homosexual behavior, i.e. females mounting females, is rare. Secondary signs such as swollen vulva, reddening of the vulvar mucosa, mucous vaginal discharge, and frequent urination are not reliable indicators of estrus. Ovulation occurs 30 hours after the onset of estrus (range 18–45 hours). Due to the low level of blood estradiol-17-β, the expression of estrus in buffaloes is very poor. Silent heat is one of the deleterious features to the reproductive performance in the buffalo. Estrus often passes unnoticed, especially in the hot and dry seasons when grass, wallowing pools and shades are in deficiency, which make the expression more dubious. The age at puberty in buffaloes is much higher than that in cattle. As in cattle, follicular growth occurs in waves in buffaloes. Two-wave cycles are most common (63.3%) followed by three wave cycles (33.3%) and a single wave cycle (3.3%). The number of waves per cycle influences the length of the luteal phase and the estrous cycle (Baruselli *et al.,* 1997). Covert or silent estrus is the single largest factor responsible for poor reproductive efficiency in buffalo. Estrus detection is a prerequisite for efficient reproductive efficiency of the dairy herd. Accurate estrus detection is essential when hand-mating to selected sires is practiced. To compensate for the lack of overt estrus behavior among females following mentioned are the methods of estrus detection:

Wall charts, breeding wheels, herd monitors and individual records: These systems are the least expensive. The key for successful use of these management aids is accurate recording of every heat beginning with the first after calving and their daily use to identify those buffaloes that are due to return to estrus.

Mount detection aids which either may be pressure sensitive devices like male chin ball markers and paint stick or paint on tail head of female.

Heat detector animals: vasectomized sexually active bulls are used to detect buffalo in heat. They are also fitted with chin ball markers.

Use of trained dogs: Dogs can be trained to detect odour associated with pheromones related to estrus in cattle and buffaloes.

Milk progesterone assay: With the help of radioimmunoassay (RIA), milk progesterone concentration can be determined. Progesterone level of < 1 ng/ml indicates an animal is in follicular phase.

Use of pedometer: Pedometer devices typically contain a mercury switch that is activated by movement of the animal. During estrus, animal is hyperactive and locomotor activity is increased which can be used as a basis for estrus detection.

Artificial Insemination (AI)

Successful AI depends on the quality of the semen, the skill of the inseminator, the accurate timing of the insemination, and the general health and condition of the female reproductive tract. The conception rate varies depending on the time at which AI is performed. Kumaresan and Ansari (2001) conducted AI after 6-12, 12-18 and 18-24 hours of estrus and the pregnancy rates were recorded to be 16.67, 28.99 and 33.33%, respectively. The bio-stimulatory effect of the teaser bull allows for a general increase in reproductive efficiency in a herd by improving the percentage of animals cycling, and by improving the pregnancy rate from 19 to 43% (Zicarelli *et al.,* 1997).

Synchronization of estrus

8 Natural or synthetic progesterone-containing devices (injections, intra-vaginal pessary, ear implants along with estradiol, PMSG and prostaglandin) have been used successfully to improve synchrony of estrus and conception rate in dairy animals. The results of single or double injection regimens with prostaglandin in cyclic buffalo are comparable with those obtained in cyclic cattle (Kamonpatana *et al.,* 1979). Synchronized ovulation and timed AI (OvSynch: GnRH, Day 0; $PGF_{2\alpha}$, Day 7; GnRH, Day 9; AI, Day 10) is a well-established reproductive management technique in cattle and buffaloes. This second administration of GnRH improves the efficiency of fixed time insemination because it synchronizes the ovulation in a short period of time. Using Ovsynch protocol in the period of transition to seasonal anestrus, obtained a conception rate of 36% (Neglia *et al.,* 2003) in buffaloes. Using the progesterone releasing intra vaginal device (PRID) regime, it is possible to synchronize estrus in cycling and non-cycling cattle/buffalo, thereby overcoming the problem of estrus detection and increasing the effectiveness of AI programme. The conception rate in riverine buffaloes was reported to range from 22.2 to 37.5% when PRID (contained progesterone and estradiol-17β) and PMSG were used (Zicarelli *et al.*, 1997; Barile *et al.,* 2001). Better results have been obtained using PRID plus PMSG and prostaglandin.

Fertility is important regardless of lactation length. Whenever insemination is planned the animal should be capable of conception. The metabolic load on modern genotypes may make this difficult and in this respect developing nutritional strategies for high-producing cows remains important. The first consideration is of negative energy balance that leads to many negative outcomes and is a major factor contributing to the pathogenesis of infertility and ultimately the economic loss. In addition, recovery from uterine inflammation and infection after calving are critical for the uterus to provide a favourable environment for establishment and maintenance of successful pregnancy. Therefore, future strategies to improve fertility focusing on the early post-partum period should be based upon minimizing the duration and degree of negative energy balance and resolving uterine infection. Reproductive management and selection for fertility have been used less for buffalo than for cattle. Poor expression of estrus behavior is the primary factor responsible for low reproductive efficiency in buffalo. Difficulties in estrus detection can be ameliorated by the use of teaser animals. In addition, artificial control of the estrous cycle has provided an efficient means of increasing reproductive efficiency.

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