Buffalo reproduction: an Indian perspective

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The increasing world buffalo population, of which over 97% is concentrated in Asia, reflect the prominent role it plays in rural economy of the region through providing milk, meat and the draft work-force. In recent decades, buffalo farming has attracted increased attention in Mediterranean and Latin American countries as well as in the US and traditionally non-buffalo rearing European countries too. The increasing interest in buffalo rearing is primarily due to more desirable composition of buffalo milk in comparison to cow milk w.r.t. high milk fat content but low in cholesterol; higher total solids, SNF, proteins, calcium, phosphorus and calories per unit. Similarly, buffalo meat is also relished more due to its lean and low cholesterol properties. India is increasingly exporting buffalo meat with no scare of transmitting the dreaded mad-cow disease. Indian buffaloes account for more than half of the world buffalo population. With their population of over 98 millions, buffaloes contribute over 56% to India's milk pool, much higher than the contribution of almost double the population of cattle.

In buffalo husbandry, reproductive performance of the species has always remained a cause of concern for the farmers as well as the veterinarians and scientists alike. Among various factors responsible for poor reproductive performance of buffalo, late maturity, long intercalving intervals, poor estrus expression - especially during summer months and seasonality of breeding activity are important (Gangwar, 1980; Janakiraman, 1988). The season of calving also influences resumption of postpartum ovarian activity. While it is generally believed that buffaloes fare poorly in reproductive performance, the issue is debatable in view of increasing buffalo population and its noteworthy contribution to milk production in India as well as the world. The current manuscript discusses problems and prospects of buffalo reproduction, as perceived by the scientists, veterinarians and farmers of the country.

Genital organs

Ovary is the primary organ controlling the success or failure of reproductive process, right from estrus expression to maintenance of gestation, process of parturition and inducing lactation for nourishing the young-one. Most studies suggest ovarian size to be much smaller in buffalo than in cow (Fahimudeen, 1975), which makes rectal palpation difficult. Consequently, the physiologically important ovarian compartments viz. the follicle and the corpus luteum are also smaller in buffalo with their consequences on reproductive performance. However, the fact that most of the females ending up in slaughterhouse are not enjoying optimum reproductive success, should not be overlooked.

The high frequency of embedded luteal structures in buffalo ovaries further precludes accuracy of per-rectal palpation, though availability of ultrasonographic monitoring facilities is helpful in arriving at an appropriate judgment of ovarian activity. This underlines the need to select heifers for larger ovarian size and luteal volume or vigour, but these remain the least emphasized options in buffalo breeding strategies which predominantly revolve around production potential. Not even the scrotal circumference of breeding bulls is often considered when they enter the breeding programme.

Puberty

In different parts of India, having diverse buffalo rearing and management practices as per agro-climatic and socio-economic conditions, age at puberty ranges from as early as 18 months to as late as 46 months. Under favourable field conditions, the first estrus may occur at 24-30 months, though the ignorant farmers usually manage heifers on bare minimal ration and supplementation of mineral mixture is rare leading to late puberty. Such heifers respond well to the initiation of mineral mixture supplementation.

Puberty is significantly affected by

- breed
- season and climate
- feeding regimen / growth rate
- disease incidence
- heat stress alleviation practices being implemented.

Body weight is considered to be an important determinant of the age at puberty and accordingly, the age at puberty is altered based on the feeding regimen available to growing heifers. Therefore, appropriate feeding regimen right from birth of the calf is emphasized for early attainment of puberty.

At the same time, desirability of too early an onset of puberty is also debatable for its consequences on production performance of buffaloes in their first lactation as well as subsequent productivity. Very young heifers may conceive but deliver smaller calves, their first lactation productivity remains low and extended service periods are also encountered. We have found that the ovaries of prepubertal heifers do respond to exogenous GnRH, but the ovarian activity is not sustained and these heifers soon relegate to acyclic status.

Seasonality

Another controversial aspect of buffalo reproduction is the existence of seasonality of reproduction. While some opine against such a phenomenon in buffaloes, others vouch for a definite seasonality of buffalo reproductive activity. Both may be correct. While a good proportion of well-fed and well-managed buffaloes may continue to cycle throughout the year, marginally managed buffaloes will relinquish the privilege of reproduction until the onset of a favourable season when thermal stress is removed and good quality fodder availability is ensured. Yet the existence of a definite seasonally alternating peak and nadir of buffalo reproductive activity is reflected in the glut of milk during winter months and acute scarcity encountered during summer. Well managed buffaloes, maintaining cyclicity and conceiving during summer months, are economically rewarding for the farmer by not only producing milk during scarcity, which fetches higher price, the price-tag of the buffalo itself gets escalated.

A closer investigation of the phenomenon in a herd would reveal that the improved management with application of heat stress ameliorative measures would be more successful in allowing cyclic buffaloes to maintain cyclicity with better pronounced heat symptoms and ovulation, though it may not always be so successful in inducing cyclicity in buffaloes in deep ovarian quiescence. Consequently, higher progesterone concentrations are reported during winter (Rao and Pandey, 1982) primarily owing to higher ovarian activity, but reports to the contrary are also available which indicate a positive correlation of progesterone with ambient temperature (Singh and Gangwar, 1991). Prolactin levels in buffalo are also influenced by temperature and photoperiod (Perera *et al*., 1989), the two main environmental variables encountered during summer months. Therefore, prolactin is also implicated in summer infertility. Heat stress results in two-fold higher prolactin concentrations in non-sprinkled acyclic buffaloes than those sprinkled with cold water for 2h during mid-day (Kaker *et al*., 1982). Prolactin antagonist - bromocriptine treatment lead to higher estradiol response (Arya and Madan, 1997), suggestive of enhanced ovarian follicular activity. Thus hyperprolactinaemia may also be implicated as a possible cause of late maturity and absence of behavioral estrus in buffalo heifers exposed to heat stress.

Further, the correlation between LH concentrations – estrual peak values as well as pulse frequency and amplitude – and ambient temperature is also reported either to be negative (Singh and Gangwar 1991) or ambiguous (Kaker *et al*., 1980). Razdan *et al.* (1981) suggested the lack of LH surge during summer as the main cause of ovarian inactivity. Diestrus pituitary responsiveness to exogenous GnRH was lower during summer than winter season (Palta and Madan, 1996).

On the whole, buffalo may be considered seasonally polyestrus short-day breeder with peak cyclic activity manifested during July to February in most parts of India - in the northern hemisphere. Perhaps it is the heat stress, rather than photoperiod, which is primarily responsible for suppression or cessation of buffalo ovarian cyclicity during summer months. Consequently, better managed buffaloes – provided with thermal comfort – continue to cycle even during summer months. The evidence of an effect of the photoperiod on seasonality of buffalo reproduction is indicated by initiation of cyclicity in summer anestrus buffaloes administered melatonin implants (Prakash *et al*., 2005). Further, tropical countries – home to the vast livestock and buffalo population – also experience nutritional inadequacies which get aggravated during summer when not only the quality of fodder deteriorates but the buffalo, with comparatively disadvantaged thermoregulatory mechanism, has to exert extra for coping with heat stress.

Nevertheless, ultrasonographic scanning of ovaries in buffaloes, declared anestrus with routine farm estrus detection practices of twice a day teaser parade, indicate the presence of cyclic luteal structures in 25-30% cases even during peak summer season. These may be considered silent estrus animals and can be suitably treated with a combination of managemental and therapeutic measures with timed insemination, though the outcome of therapeutic interventions does not remain as rewarding as during the season of peak breeding activity.

Oestrus

The husbandry practices in vogue in most of the buffalo rearing countries in the region, including India, are such that the single or few buffaloes kept by individual farmers remain tied throughout the day and night with little physical interaction for behavioural manifestations. Overall poor behavioural expression of estrus, short estrus duration, more intense manifestation during night hours, coupled with inadequacies of farm estrus detection schedule would influence reproductive performance (Gill *et al*., 1973). The average duration of estrus in buffaloes is 14-16 h, far less than in cattle. Incidence of undetected estrus is more frequent during early postpartum period (Yashpal *et al*., 1999, 2000), which leads to increased service period and days open. Most farmers rely on vocalization as the sign of estrus in a buffalo, while other manifestations such as mucosal vaginal discharge, swollen vulva, frequent urination, mounting behaviour and standing to be mounted are often overlooked (Kumar *et al*., 2007). In addition, the wider interval between estrus and ovulation in buffaloes, can partly be held responsible for low conception rates. It is important that correlation of LH surge with estrus and ovulation is established so that high pregnancy rates are achieved with suitably timed inseminations. In buffaloes, incidence of LH peak is highly variable in relation to estrus or estradiol peak. Batra and Pandey (1983) reported an average interval of 14.8 h from estradiol peak to LH peak with 4h duration of the LH peak. Yet, in some animals, LH peak was detected as late as near the end or 24 h after the end of estrus. In Murrah heifers (Kumar *et al*., 1991), LH peak occurred 8 h after onset of estrus and peak values were sustained for 12 h. Similar wide range is noted in Ovsynch treated buffaloes which ovulate over a long interval of 8 to 60 h after second GnRH.

Buffaloes with suprabasal progesterone concentrations at AI do not conceive, and this high progesterone is attributed to incomplete regression of CL, leading to infertility or repeat breeding (Panchal *et al*., 1992). Maximum concentrations of progesterone during the luteal phase are correlated to maximum size of the CL. Weight of the CL has also been found positively correlated with blood progesterone profiles in buffaloes (Shah and Mehta, 1992) and luteal regression was accompanied with decrease in weight of CL with low progesterone concentrations and an increase in estradiol.

Gestation

The gestation period of buffaloes is approx. 310 to 330 days, average for Murrah being 315 days and longer - 330 days, for Swamp. Wallowing and fighting habits of buffaloes make its gestation more vulnerable to the incidence of uterine torsion than cattle. The organized buffalo farms are almost free of uterine torsion. Similarly, the incidence is very high in some localities yet almost non-existent in cows at the same location. The steep slope of village wallowing ponds results in higher incidence. Therefore, adoption of other methods of heat-stress alleviation like misting and showering facilities, help prevent exposure of gravid buffaloes in their late gestations to possibility of uterine torsion. The incidence of other peripartal complications such as rupture of prepubic tendon, fetal mummification, maceration, abortion, retained placenta etc. is not different from what is generally reported in cattle. However, low mineral status of buffaloes not fed balanced ration, coupled with high estradiol induced flaccidity of musculature, may predispose buffaloes to antepartum vaginal prolapse.

During pregnancy, ovarian follicular activity continued in wave pattern though gestational ovulations were rarely recorded. Inhibitory influence of the CL of pregnancy was evident on follicular development and dominance in ipsilateral ovary. Overall, the mean number of developing follicles was more in non-pregnant buffaloes than pregnant ones. Dominant follicles in pregnant buffaloes tended to be smaller than DFs in non-pregnant females.

Post partum uterine involution

In Nagpuri buffaloes, non-gravid uterine horn involuted in 10.73±0.35 days, while the gravid horn took 23.57±0.96 days, while Agarwal *et al.* (1978) found involution in 45 days for previously gravid horn and 30 days for non-gravid horn in pluriparous buffaloes slaughtered at 15 days intervals. Histologically, endometrium was re-established by 32 days postpartum. Ultrasonographic study of uterine involution in buffaloes suggested completion of uterine involution by day 45 postpartum (Lohan *et al.,* 2004). A significant effect of season of calving on uterine involution has also been reported Chauhan *et al*. (1977). Ahmad *et al*., (1985) reported that out of 25% postpartum buffaloes which showed delayed uterine involution on per rectal palpation, 80% had infected uterine fluid. $PGF_{2\alpha}$ given within 24h to one week after parturition shortened the interval to complete uterine involution and improved subsequent fertility, administration of GnRH later in the puerperium (~ day 15 to 20) was also beneficial in enhancing postpartum fertility of dairy buffaloes.

Post partum ovarian activity

Long inter-calving intervals of 400 and 600 days - one of the most commonly cited reasons for poor reproductive efficiency of the species - are ascribed to suboptimal nutritional, seasonal and managerial factors. Apart from deficiency of quality feeds and fodders for meeting high energy and protein requirements during early lactation, non-supplementation of deficient macro and micro minerals in animals' ration is another important cause of suppressed cyclicity. Strong mothering instinct of the species and wide-spread practice of suckling for milk-letdown in buffaloes exert further negative impact on resumption of postpartum cyclicity. Generally, the first ovulation in river buffaloes does not occur before 45 days post partum, but may be delayed beyond 90 days when the calf is suckling. The season of calving also influences resumption of postpartum cyclicity with extremely longer calving intervals usually seen in buffaloes calving towards the end of the breeding season.

Based on per-rectal palpation alone in post-partum buffaloes, Singh *et al.* (1979) reported the mean interval of 26.90±0.72 days for initiation of follicular development and 37.78±2.01 days for first ovulation, though the interval to first postpartum estrus was 76.96±4.62 days. Resumption of acylic ovarian activity during the second month postpartum was also evident with 53.6% buffaloes having luteal milk progesterone concentrations (Yashpal *et al*., 1999). The number of animals experiencing first postpartum estrus within 30 days was 7.7%, 31% between 30-60 days, another 31 % by 90 days and rest 30% animals took over 90 days to first postpartum estrus. However, low (18%) conception rate to first estrus within 45 days postpartum increased to 48% between 46-90 days and 63% in buffaloes exhibiting first postpartum estrus after 90 days of calving. Admittedly, a large proportion of otherwise cyclic females remain unidentified in the absence of either marked estrus symptoms or inefficiency of estrus detection regimen. We found about 25% of anestrus animals to be actually cyclic. Higher incidence of silent estrus is reported during summer months with yearly average of 37% (Prakash *et al.,* 2005).

A transient rise in milk progesterone concentrations prior to first postpartum estrus indicate silent ovulations associated with short luteal phases before establishment of normal cycles (Pahwa and Pandey, 1983; Yashpal *et al*., 1999). In addition, characteristic of poor estrus expression leads to failure to detect estrus in early postpartum (Agarwal and Purbey, 1983). On the other hand, it is also reported that the first postpartum estrus may be non-ovulatory in a high proportion (19%) of buffaloes (Suthar and Kavani, 1992).

Reproduction management

The production potential of buffalo is not utilized optimally due to reproductive inadequacies, primarily attributed to late attainment of puberty, seasonality and long postpartum anestrus leading to long calving intervals. This provides an opportunity for researchers and veterinarians to address these deficiencies for harnessing better productive output from buffalo. A simple, inexpensive test for routine diagnosis of estrus is warranted so that buffaloes are inseminated at optimal time. A reliable protocol for successful estrus induction in buffaloes is also elusive. If the protocol ensures synchronized ovulations, the problems of sub-estrus / silent estrus and associated estrus detection, can also be overcome. The use of ultrasonography for monitoring ovarian picture and follicular dynamics has greatly enhanced correct diagnosis as well as monitoring and prediction of response to different therapeutic interventions for improving reproductive efficiency of buffaloes.

In this regard, pre-treatment ovarian status was related to outcome of the 'Ovsynch' treatment, being successful in buffaloes with at least one large follicle in the ovaries at the time of start of the treatment irrespective of the cyclic status of ovaries (Rohilla *et al*., 2005). Overall, the application of Ovsynch and Ovsynch-Plus protocols in peripubertal heifers and postpartum buffaloes induced ovulations in 58% of treated females, with 28% conception rate at induced estrus (Sharma *et al*., 2004a; Singh *et al*., 2004, 2005). Norgestomet ear implants without eCG failed to induce estrus in anestrus buffaloes even during the breeding season (Dalal *et al*., 1996), though a higher dose of norgestomet with eCG resulted in acceptable estrus and fertility response even during summer season (Kundu, 1998). As in cattle, PGF2α causes rapid luteolysis in subestrus buffaloes, accompanied by accelerated growth of dominant or the second largest follicle, which culminates into ovulation with acceptable fertility (Sharma *et al*., 2004b). Continuous infusion of naloxone increased the mean concentrations, pulse frequency and pulse amplitude of LH in anestrus and cyclic buffaloes, as compared to untreated animals (Chandrasekhar and Madan, 1997) and naloxone treated anestrous buffaloes exhibited estrous average 25 days after treatment (Palta *et al*., 2000).

These results indicate that while patterns of follicular growth can be manipulated hormonally in anestrous buffaloes, CL formation and its functional performance may remain sub-optimal to sustain cyclicity or conception. The early regression of corpus luteum developed in response to Ovsynch treatment remains a dilemma and requires studies on the functional characteristics of these corpora lutea, both through establishing the hormonal profiles in Ovsynch treated buffaloes, as well as by in vitro studies on corpus luteum function. Nevertheless, the luteal insufficiency was recognized and protocols need to be recast for yielding more robust corpora lutea following first GnRH, which could be lysed at predetermined times for greater synchrony of induced estrus to allow timed inseminations.

In repeat breeder buffaloes, we found significantly improved conception rates when normal cyclic as well as repeat breeder buffaloes were given progesterone depot supplements at critical stages post-insemination (Kumar *et al*., 2003). Arora and Pandey (1982) reported that the LH peak on the day of insemination was either absent or lower in repeat breeding buffaloes as compared to the buffaloes which conceived.

Artificial insemination

The technique of AI has earned disrepute in buffalo husbandry with low adoption rate by farmers, who still prefer natural mating. Ignorance about benefits of AI, inability to identify appropriate stage of estrus, wide range of interval to ovulation, non-availability of quality frozen semen and inadequate infrastructure, are some of the factors which compromise conception rates to AI. Nevertheless, the technique is being pursued and getting increasing attention in the recent years due to heightened interest in Murrah breeding and low availability of elite bulls for natural mating (Singh *et al*., 2006b). The techniques and infrastructure for semen freezing have been scaled up leading to better conception rates. With trained layman inseminators using quality frozen semen, conception rates exceeding 55% are not uncommon while some are approaching CR of 70%, previously limited to 17-33% (Kumaresan and Ansari, 2001). For buffalo AI, the theory of AM-PM breeding, as practiced in cattle, may not be successful. Later inseminations, approximately 20 h after first noticed estrus activity, are reportedly more successful in ensuring better fertility.

Male reproduction

Buffalo reproduction is reported to be suboptimal in comparison to cow and is further adversely affected during summer season, both in the female as well as the male. Reproductive ability of male buffalo bull is considerably lower in comparison to cow bull with respect to semen output, quality and freezability as well as libido. This is amply indicated by the basic information on testicular function of buffalo bulls. The duration of seminiferous epithelial cycle (16 days), spermatogenesis (75 days), spermatocytogenesis (27.6 days) and spermiogenesis (21.7 days) for buffalo bulls have been reported (Sharma, 1977). Determination of epididymal sperm reserve is helpful to form a guideline for the frequency of ejaculation, which averaged 36 billion, out of which the caput and cauda epididymis contained 24.36 and 53.67 per cent sperm, respectively. Daily sperm production rate in buffalo bulls with an average paired testes weight of 280 gm, was 40.4±0.18 billion sperm per day with efficiency of sperm production being $14.5x10^6$ sperm per gram of testicular parenchyma per day, irrespective of testicular weight. Average DSPR values in buffalo bulls are quite low as compared to cow bulls, boar, ram and stallion. The contributing factors for such low values in buffalo bulls are small size of the testes, less number of spermatogenic cells in the seminiferous tubules and long duration of SEC. Sperm production rate in different species can be meaningfully compared by considering the DSPR values relative to epididymal sperm reserve, because the differences among the species in the ratio of sperm in cauda epididymides to DSPR may possibly have evolved concurrent with differences in the length of the breeding season, duration of estrous and mating behaviour of the males. In the buffalo bull, this ratio is 1:9, whereas in stallion, ram, bull and boar it was calculated to be 1:10, 1:12, 1:5 and 1:6, respectively. So, the low DSPR and extra-gonadal sperm reserve along with less sperm production per gram of testicular parenchyma and too frequent use of the males in this species can be potentially responsible for low conception rate in buffaloes.

The Society for Theriogenology guidelines for breeding soundness examination of cow bulls emphasizes the importance of scrotal circumference in bull selection. Scrotal circumference is a simple and reliable parameter for determining the reproductive capacity of breeding bulls. Most importantly, scrotal circumference with its consequences is highly heritable. Scrotal circumference of mature breeding buffalo bulls averaged 34.17±0.23 cm (Malik, 2002; Singh *et al*., 2009), a diameter that is attained as early as 15-30 months in various European breeds of cow bulls.

In buffalo semen cryopreservation a large proportion of semen ejaculates, particularly during summer months, are rejected due to a high incidence of post-thaw backward motility of sperm cells. It was found that the phenomenon was associated with level and stage of glycerol addition in the extended semen (Singh *et al*., 2006a). When glycerolization was done at room temperature during initial stage of semen dilution, the incidence of post-thaw backward motility of spermatozoa was reduced to negligible levels as compared to 40-60 per cent in some ejaculates which were glycerolized after cooling to 5˚C. Comparison of ejaculate freezing rates over one year period each with the two protocols of glycerolization, glycerolization after cooling to 5˚C and the modified protocol with glycerolisation at room temperature, revealed that the modified protocol reduced rejection rate of ejaculates by 20 per cent. In this way, freezing of 20 per cent more ejaculates greatly enhanced frozen semen production.

Overall, buffalo reproduction may in fact not be as gloomy as it is often portrayed and inspite of various constraints of climate, nutrition and routine husbandry practices, buffaloes can maintain cyclicity under good management and appropriate therapeutic interventions. The interest and confidence of farmers in the Artificial Insemination technique is increasingly rapidly with assurance of high conception rates. This is leading to renewed interest of local farmers in buffalo breeding for conservation and propagation of superior Murrah germplasm in view of its high demand worldwide.

References

Agarwal KP et al. Postparturitional changes in the uterus of buffalo cows. *Indian J Anim Sci*, v.48, p.492-503, 1978.

Agarwal SK, Purbey LN. Estrus behaviour and its relation to conception in rural buffaloes. *Indian Vet J*, v.60, p.631-636, 1983.

Ahmad R, Amin SM, Kazimi SE. Studies on the bacterial causes of delayed uterine involution in postpartum buffaloes. *Pakistan Vet J*, v.5, p.168-170, 1985.
Arora RC, Pandey RS. Plasma concentrations of progesterone, estradiol and LH in relation to repeat breeding buffaloes. *Anim Prod*, v.34, p.139-144, 1982.
Arya JS, Madan ML. Hyperprolactinaemia: a possible cause of late maturity in buffalo heifers. *Indian J Anim Prod Manag*, v.13, p.193-197, 1997,
Batra SK, Pandey RS. Luteinizing hormone and estradiol-17? in blood plasma and milk during estrous cycle and early pregnancy in Murrah buffaloes. *Anim Reprod Sci*, v.5, p.247-257, 1983.
Chandrasekhar T, Madan ML. () Plasma levels of luteinizing and follicle stimulating hormones in naloxone-treated subestrous buffaloes. *Indian J Anim Sci*, v.67, p.16-18, 1997.
Chauhan FS, Singh N, Singh M. Involution of the uterus and cervix in buffaloes. *Indian J Dairy Sci*, v.30, p.286-291, 1977.
Fahimuddin M. *Domestic water buffalo*. New Delhi: Oxford and IBH Publ, 1975. p.236.
Gangwar PC. Climate and reproduction in buffaloes - a review. *Indian J. Dairy Sci*, v.33, p.4, 1980.
Gill RS, Gangwar PC, Kooner DS. Studies on estrus behaviour of buffaloes. *Indian J Anim Sci*, v.43, p.472-475, 1973.
Janakiraman K. Some aspects of reproductive problems in buffaloes. *In*: World Buffalo Congress, 2, 1988, New Delhi. *Proceedings* … New Delhi: WBC, 1988. v.2, part 1, p.264-270.
Kaker ML, Razdan MN, Galhotra MM. Serum LH concentration in cyclic buffaloes. *J Reprod Fertil*, v.60, p.419-424, 1980.
Kaker ML, Razdan MN, Galhotra MM. Serum prolactin levels of Noncyclic Murrah buffaloes (Bubalus bubalis). *Theriogenology*, v.17, p.469-474, 1982.
Kumar P, Jindal R, Rattan PJS. Plasma hormone profiles during estrous cycle of Murrah buffalo heifers. *Indian J Anim Sci*, v.61, 382-385, 1991.
Kumar R, Phogat JB, Singh I, Kumar B, Singh U, Sethi RK. Efficacy of post-insemination progesterone supplementation for enhancement of fertility in buffaloes. *Bubalus bubalis*, v.3, p.76-82, 2003.
Kumar R, Singh U, Singh I, Phogat JB. Reliability of estrus behavioral signs for estrus detection in hormone treated post partum summer anestrus buffaloes. *Haryana Vet*, v.46, p.28-32, 2007.
Kumaresan A, Ansari MR. Evaluation of conception rate in buffaloes (Bubalus bubalis) with reference to semen quality, stage of estrus and inseminator. *Indian J Anim Sci*, v.71, p.144-145, 2001.
Lohan IS, Malik RK, Kaker ML. Uterine involution and ovarian follicular growth during early postpartum period of Murrah buffaloes. *Asian-Aust J Anim Sci*, v.17, p.313-316, 2004.
Malik J. Thesis (PhD) - CCS Haryana Agricultural University, Hisar, India, 2002.
Malik RK, RK Sharma, Singh P, Singh JK, Singh I. Follicular dynamics during early postpartum period in buffaloes. *In*: National Symposium of ISSAR, 21, 2005, Jammu. *Proceedings* … Jammu: The Symposium, 2005. p.198.
Pahwa GS, Pandey RS. Gonadal steroid hormone level in blood plasma and milk of primiparous and multiparous non-pregnant buffaloes. *Theriogenology*, v.19, p.491-505, 1983.
Palta P, Chandrashekhar T, Prakash BS, Madan ML. Effect of naloxone on GnRH-induced LH and FSH release in buffalo. *Indian J Exp Biol*, v.38, p.390-392, 2000.
Palta P, Madan ML. Seasonal variations in the hypophyseal responsiveness to GnRH in cycling buffalo (Bubalus bubalis). *Asian Aust J Anim Sci*, v.9, p.711-714, 1996.
Panchal MT, Dhami AJ, Mehta VM, Kodagali SB. Plasma progesterone profile of fertile and infertile oestrus cycles in repeat breeding buffaloes. *Indian Vet J*, v.69, p.766-767, 1992.
Perera KS, Gwazdauskas FC, Akers RM, McGilliard ML. Effect of supplemental light on growth, prolactin, progesterone and luteinizing hormone in water buffalo (Bubalus bubalis). *Int J Biomet*, v.33, p.89-94, 1989.
Prakash S, Sarkar DP, Vijay P, Mishra A, Meyer HHD. Postpartum endocrinology and prospects for fertility improvement in the lactating riverine buffalo and yak. *Livest Prod Sci*, v.98, p.13-23, 2005.
Rao, LN, Pandey RS. Seasonal variations in plasma progesterone concentrations in buffalo cows. *J Reprod Fertil*, v.66, p.57-61, 1982.
Razdan MN, Kaker ML, Galhotra MM. Serum luteinizing hormone levels of noncycling buffaloes (Bubalus bubalis). *Indian J Anim Sci*, v.51, p.286-288, 1981.
Rohilla N, Singh U, Sharma RK, Singh I. Ultrasonic ovarian status in summer anestrus postpartum Murrah buffaloes. *Indian J Anim Reprod*, v.26, p.95-98, 2005.
Shah RG, Mehta VM. In vitro progesterone synthesis and secretion rate in corpus luteum of Surti buffaloes. *Buffalo J*, v.8, p.271-276, 1992.
Sharma AK. Thesis (PhD) - Haryana Agricultural University, Hisar, India, 1977.
Sharma RK, Singh I, Singh JK, Sethi RK. Ovsynch-Plus A new protocol for synchronized estrus induction and fixed time insemination in peripubertal Murrah buffalo heifers. *In*: National Symposium on Livestock Biodiversity (NBAGR), 2004, Karnal. *Proceedings* … Karnal: The Symposium, 2004a. p.199.
Sharma RK, Singh I, Singh JK. Growth and regression of ovarian follicles and the corpus luteum in subestrus

Murrah heifers undergoing prostaglandin induced luteolysis. *In*: World Buffalo Congress, 7, 2004, Makati, Philippines. *Proceedings* … Makati, Phillpines: WBC, 2004b. p. 68.

Singh I, Sharma RK, Mehrara KL, Singh JK. Ovarian response and pregnancy rate in summer anestrus buffaloes receiving 'Ovsynch' and 'Ovsynch-Plus' treatments. *In*: National Symposium of ISSAR, 21, 2005, Jammu. *Proceedings* … Jammu: The Symposium, 2005. p.206.

Singh JK, Sharma RK, Singh I, Singh S Ovarian follicular dynamics during estrus induction and subsequent fertility of true anestrus peripubertal Murrah heifers receiving 'Ovsynch' and 'Ovsynch-Plus' treatments. *In*: National Seminar on Economic feeding and rearing of buffaloes (IGFRI), 2004, Jhansi. *Proceedings* … Jhansi, The seminar, 2004. p.37.

Singh N, Chauhan FS, Singh M. Postpartum ovarian activity and fertility in buffaloes. *Indian J Dairy Sci*, v.32, p.134-139, 1979.

Singh N, Gangwar P. Hormonal profiles in normal and anestrous buffaloes during summer. *Indian Vet J*, v.68, p.993-994, 1991.

Singh P, Singh I, Singh S, Sharma RK. Initial stage glycerolisation prevents the incidence of backward sperm motility during cryopreservation and increased buffalo semen freezability. *Indian J Anim Sci*, v.76, p.777-779, 2006a.

Singh P, Singh I, Singh K, Singh S, Phulia SK. Relationship of age and body weight with scrotal circumference in Murrah buffalo bulls / male Murrah buffalo. *Indian J Anim Sci*, 2009. (In press)

Singh R, Singh I, Singh P. Erosion of elite Murrah buffalo germplasm from its home tract. *Indian Buffalo J*, v.4, p.62-65, 2006b.

Suthar BN, Kavani FS. Occurrence and nature of first postpartum estrus in Mehsani buffaloes. *Indian J Anim Reprod*, v.13, p.161-164, 1992.

Yashpal, Singh I, Phogat JB, Singh U, Khanna S, Singh S, Chander S. Whole milk progesterone indicates earlier resumption of postpartum ovarian cyclicity in buffaloes. *In*: National Symposium on Biotechniques in Optimizing Fertility in Farm Animals, PAU, 1999, Ludhiana. Ludhiana: The Symposium, 1999. abstr. 63.

Yashpal, Singh I, Singh U, Phogat JB. Reproductive parameters of postpartum Murrah buffaloes. *Haryana Vet*, v.39, p.20-27, 2000.