# Recent trends in Buffalo reproduction

K.P. Agrawal

*K.P. Agrawal*
*National Coordinator*
*National Agricultural Technology Project*
*Indian Council of Agricultural Research*
*KAB – II, Pusa Campus*
*New Delhi – 110 012*

The buffaloes are the mainstay of Indian dairy industry, contributing over 50% of the total milk produced in the country besides being used for meat and draft purposes. The productive and reproductive efficiency especially of animals are complimentary to each other. Low reproductive efficiency in general and buffaloes in particular remains a major economic problem globally, and its incidence is higher in our country. Climatic stress, nutritional deficiency, improper management and lack of disease prevention and control measures are the main attributing factors.

Reproductive efficiency (fertility) can be defined as ability of an animal to produce the youngone. But the interest of an animal breeder is not served simply if an animal is capable to produce the youngone. The main interest of animal breeder is to achieve more youngones in lifetime, reduced mortality and healthy and superior youngones. To achieve the above goal, a knowledge of normal reproductive processes/ behavior and its management, modern reproductive tools to augment reproductive efficiency (fertility) and reproductive constraints and remedies becomes obvious. This paper describes different approaches to augment reproduction in buffalo.

EFFECT OF CLIMATE ON REPRODUCTION: A determining factor in the production and reproduction of farm animals all over the world is environment. Inborn production potentialities can be severely limited by exposure to adverse climatic conditions. Season affects the breeding efficiency in case of buffaloes. There is a tendency to have better performance during the cool months. Several workers reported 70 to 80% conception in buffaloes between July to February. A lower number of services per conception are needed during this breeding season than other breeding season (March to June). Results of several studies conclude that buffaloes are sexually activated by decreased day length and temperature. Season of calving affects lactation production, lactation length and calving interval. Winter calvers produced more milk in a lactation as compared with those calving in rainy season. Least calving interval is, however, in those who calved in rainy season. Oestrogen activity of some fodders also influence seasonal activity.

EFFECT OF NUTRITION ON REPRODUCTION: Adequate nutrition is the prerequisite for proper functioning of the reproductive system in animals. Under feeding, over feeding, protein and vitamin deficiencies, imbalance of trace elements result in various reproductive abnormalities both in male and female. Poor nutrition during early life in female retards the onset of puberty. In parous cows, it leads to anoestrus, anovulation and metabolic disorders. During early pregnancy, moderate to severe under nutrition may lead to embryonic mortality and/or abortion. During late pregnancy it has an adverse effect on calf birth weight. A poor body condition score (BCS) at calving adversely affects milk production and fertility characterized by prolonged post-partum oestrus interval, reduced conception rate and more services per conception. The nutrient deficiencies, excesses and imbalances during initial period of lactation can lead to several disorders, which affect the post-patrum reproduction. A very low protein diet can cause cessation of oestrus, if fertilization occurs, foetal death or the birth of premature and weak offsprings results. The other common reproductive problem in lactating cows is cystic ovaries. High levels of grain feeding and plant oestrogens are associated with increased frequencies of cystic follicles. The plane of nutrition also has an influence on the performance of bulls. Ration containing low protein affects adversely semen volume and quality. Continued feeding of low protein ration, besides affecting quality and quantity of semen also affects sex libido.

MANAGEMENT OF REPRODUCTIVE PROCESSES ASSOCIATED WITH NUTRITIONAL AND CLIMATIC FACTORS: Studies conducted at Veterinary College, Mathura in 1960's concluded that it is possible to breed buffaloes through out the year under suitable management conditions although signs of heat are depressed during summer months. Breeding was restricted from 1st April to 30th June, the hottest part of the year. Of the buffaloes kept in sheds during day and in open paddocks during the night, 90 percent came into heat vs 98 percent of those cooled by sprinkling with water three times a day. The corresponding figures for conception rate were 60.00 percent vs 79.60 percent.

Oestrus detection which is one of the main causes of apparent anoestrus in the tropics and subtropics is another constraint. In the buffalo homosexual tendency is usually absent and oestrus signs are weaker even during the height of breeding season (autumn and winter), while during the summer months the symptoms become weaker still. Moreover, use of teaser bulls is of doubtful value in detecting oestrus as they lose libido when exposed to direct and indirect effects of solar radiations in summer season. Inadequate oestrus detection leads to increased days open, irregular cyclicity, long return intervals, higher incidence of repeat breeding, not responding to treatment for infertility, poor breeding efficiency and low economic return.

Other factors which adversely affect oestrus exhibition after calving include almost total lack of weaning practice (even in most organized farms) and inadequate supply of nutrients to the dam. Suckling is one exteroceptive stimulus that leads to extended and variable period of anoestrus and anovulation following calving. It also increases the incidence of spontaneous short cycles, associated with short duration and reduced intensity of oestrus. The adverse effects of suckling are further aggravated during summer months.

**ARTIFICIAL INSEMINATION (AI):** One of the greatest sources of failure in artificial insemination is the inability to recognize oestrus display. With frozen semen, the need to inseminate close to ovulation is even more imperative because of the limited survival of the spermatozoa. Where it is possible to extend the life of spermatozoa and to package them in such a way as to retain them in the reproductive tract in a viable state for several days, then insemination would need not to be timed to ovulation, and a more relaxed regime could be established. Efforts are being made for microencapsulation of spermatozoa. In this technology, which is used extensively for storing cell-lines, spermatozoa in an alginate solution are extruded in minute drops of suspending medium into a calcium solution that changes the alginate from sol to gel phase. The gelatinous capsules are then encased in plastic shells, e.g. poly-L-lysine, and the calcium ions are withdrawn with EGTA returning the contents of the capsule to a sol. Spermatozoa will survive this treatment. The other problem of retention of capsules in the female reproductive tract, is their timed breakdown, releasing spermatozoa for fertilization at the appropriate time. The process is not simple and requires further investigation. First there is a need to investigate how to avoid the stress of the encapsulation process or to protect the spermatozoa through it. Secondly, maintenance of viability of the cells at body temperature needs to be investigated. This is achieved in the female tract in the short term over several days depending on species, and on a longer term before ejaculation in the cauda epididymidis for upto 1-2 weeks. Certain species of bats store spermatozoa in the uterus over winter, and birds have sperm storage organs in the lower vagina that maintain viability for several days or weeks at temperatures over 40°C. The challenge is to discover how nature achieves this phenomenon and to harness it to the technological objective. Thirdly, the retention of the capsules in the uterus or vagina requires attention. Normally, a foreign body of this sort is eliminated rapidly through the cervix and will be washed out of the vulva by the flow of fluids. The capsule surface will need to be fabricated of a material that will attach to the mucosa, but will breakdown over a suitable time-scale to allow release of spermatozoa.

In contrast to bull spermatozoa, buffalo spermatozoa are more susceptible to hazards during freezing. The osmolarity of seminal plasma of buffalo bull is different than the cattle seminal plasma, hence, for cryo-preservation of buffalo semen, a different type of extenders and techniques are required. The techniques of freeze-drying, vitrification and in-vitro culture of testicular tissue are newer areas to work.

**INDUCTION AND SYNCHONIZATION OF OESTRUS (MANIPULATION OF BREEDING CYCLE):** One of the greatest impediments for increasing reproduction performance is the poor _expression of oestrus signs in buffalo-cows. Controlling the oestrous cycle and resorting to fixed time AI would provide a means for circumventing the problem of oestrus detection. For satisfactory pregnancy rates in an embryo transfer programme, the embryo must be placed in an environment that simulate the one from which it is removed. Therefore, synchronization of oestrus in donor and recipient in each set of experiment is considered essential to obtain the best results.

Two alternate approaches are used for induction and synchronization of oestrus. The first is by artificially extending the luteal phase (by using progestational compounds) and second by inducing the demise of the corpus luteum (by using prostaglandins and their analogues). Progestogen and prostaglandin in combination are also used. During the non-breeding and anoestrus season, treatment of the females to induce oestrus and ovulation is similar to that described for the breeding season. Progestogens with some possible adjustment in PMSG and/or FSH are used for anticipated lower response. $PGF_2$ alpha in double dose schedule (10-11 days interval) can be used to cover those animals who do not have functional CL at first injection.

**SUPEROVULATION:** Superovulation (multiple ovulation) is one of the major reproductive technologies for rapid genetic improvement of the Livestock.

Unfortunately, high variability in the ovarian follicular response to gonadotropin stimulation continues to be the major problem. Considerable inconsistency in characterization of follicular dynamics after superovulation treatment has been observed in Zebu cattle and buffaloes as compared to results observed in Bos taurus. The response in buffaloes as compared to Zebu cattle is poor and reasons are numerous, such as difficulty in detecting oestrus, least selection pressure for fertility, genetic background, higher stress exposure, lower feed availability and disease situations. The other reasons are: 1) population of primordial follicles is only one tenth that of cattle, 2) number of antral follicles is lower at all stages of the oestrous cycle, 3) the incidence of deep atresia is higher, 4) the shift from small to large follicles is slower and 5) higher incidence of ovulation failure.

**FACTORS AFFECTING SUPEROVULATORY RESPONSE:** A number of factors affect the superovulatory response, viz: source of gonadotropins, stage of oestrous cycle and breed.

**Source of gonadotropins:** Pregnant mare serum gonadotropin (PMSG) is a glycoprotein having both FSH and LH activity. Its biological life is approximately 40 hours and it has been shown to persist for up to 10 days in the circulation. Also it has been shown that the ratio between FSH and LH varied between different batches. The long half-life of PMSG and the high levels of LH in some batches resulted in unovulated follicles, fertilization failure, reduced embryo quality and poor recovery rate. These problems are attributed to the high levels of oestrogen and progesterone in the blood that in turn effect sperm transport and capacitation and delayed transport of embryos through the oviduct into the uterus. Recently, these problems are overcome with the development of Neutra – PMSG, a monoclonal antibody, which is injected at the time of the first insemination. Follicle stimulating Hormone (FSH – P) is derived from the pituitaries of domestic animals. FSH – P also contains varying levels of LH depending on the batch. Its biological half life is estimated to be 5 hours. FSH – P has been the most widely used hormone to induce superovulation. Folltropin – V is a highly purified porcine pituitary extract containing very little amount of LH. Because of low levels of LH, the most of the detrimental effect, viz: fertilization failure, poor embryo quality and embryo recovery are minimized and thus resulted in large number of transferable embryos. The stimulation of preantral follicles by priming with gonadotropins to prevent them from becoming atretic may increase the pool of growing follicles prior to superovulation thus increasing the ovulation rates and number of transferable embryos. Priming dose of FSH (20 mg folltropin – V) is given at the beginning of the cycle (day – 3 of the cycle). Gonadotropin releasing hormone (GnRH) agonists are being utilized to improve synchronization of ovulations in superovulation experiments. This is based on down regulation of pituitary gonadotropins by a GnRH agonist (Deslorelin) resulting in follicular regression. Exogenses FSH is then used to stimulate uniform follicular growth followed by exogenous LH to bring about close synchrony in ovulation. The approach also eliminates oestrus detection of donors and reduces the number of inseminations as donors can be inseminated once at a set time in relation to the time of exogenous LH administration.

**Stage of Oestrous Cycle:** Administration of exogenous gonadotropin at different stages of the oestrous cycle affects the superovulatory response. Injection of PMSG (1500-2000 i.u.) during follicular phase (15-16 days of oestrous cycle) exhibits oestrus 2-6 days after PMSG injection. No hormonal treatment is required to induce ovulation. In the case of luteal phase stimulation, PMSG is injected between day 10 and day 12 of the oestrous cycle. Prostaglandin is injected 48 to 72 hours after PMSG injection to induce oestrus. Most of the studies revealed luteal phase stimulation more effective in terms of ovulation rate and embryo recovery as compared to follicular phase stimulation. The superiority of mid luteal phase stimulation is attributed to the fact that a second wave of follicular development begins approximately 8 to 10 days post ovulation. The follicle growth occurs in 2, 3 or even 4 waves during each oestrous cycle and that a dominant follicle emerges 6 days post ovulation. Poor embryo recovery rates in some donor cows, superovulated during luteal phase, have been attributed to the presence of a dominant follicle. Different methods/hormonal agents (oestradiol valerate, HCG) have been used to eliminate the effect of dominant follicle on superovulatory response.

**Breed:** Different breeds of donor cows respond differently to superovulatory treatments. Bos Taurus cattle need more FSH than Bos indicus cattle. Dairy cows respond differently as compared to beaf cows.

**EMBRYO TRANSFER:** The application of artificial insemination technique has provided considerable opportunity to improve the genetic potential of the livestock by using semen from proven males. The embryo transfer technique permits exploitation of superior female genotype, giving more offsprings from the same genetic donor than would arise under normal conditions of breeding. Embryo transfer technology in buffaloes has limited success due to several reasons, viz: low reproductive efficiency, poor superovulatory response, very low primordial follicle population, high incidence of deep atresia etc. Conception in recipients following embryo transfer is very low (16%).

Application of embryo transfer includes its use in genetic improvement through selection of parents, multiplication and mechanical manipulation of its genetic components, preservation, conservation of threatened breeds, more rapid propagation where a limited number of individuals exist, production of specific pathogen free (SPF) population and

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