Recent trends in reproductive management

G. Ajithkumar,B. Bibin Becha and T. Sreekumaran

Dr. G. Ajithkumar, MVSc, DRD, CPH, PGJMC.,
Dr. B. Bibin Becha, MVSc.
&
Dr. T. Sreekumaran, BVSc, MSc (Vet), PhD.
College of Veterinary and Animal Sciences, Mannuthy

In order to exploit the maximum genetic potential of a dairy animal, it has to reproduce optimally. A normal fertile animal should calve by 2 to 2½ years of age and then by every year with a service period of 60 days and an A.I. index (number of inseminations per conception) of 1.5.

Delayed puberty, true anoestrus, postpartum anoestrus, suboestrus, ovulatory disturbances, cysticovarian degeneration, uterine infections and repeat breeding are of more concern in contributing to infertility in bovines. In recent years, more attention is focussed on manipulating the reproductive endocrinology for augmenting fertility in bovines.

Correction of functional disorders of ovary, synchronization of oestrus and correcting uterine infection are of great importance in gynaeco-clinical therapy. In modern practice, hormonal preparations containing Gonadotropin Releasing Hormones (GnRH), gonadotropins, steroids and prostaglandins are widely used for augmenting fertility.

Anoestrus

Crossbred heifers should attain sexual maturity by about 15 to 18 months of age. Delayed puberty in heifers results in economic losses despite of their better health status, optimum body weight and age for attainment of puberty. Hence it is imperative to use exogenous hormones to bring them into oestrus within the stipulated time.

A thorough investigation keeping in view of various agro-climatic conditions and deficiencies of blood constituents backed by diagnostic aids may be useful in tackling the serious problem of delay in maturity and subsequent fertility. Mostly a deficiency if occurs, it will be marginal and difficult to assess clinically. The response to exogenous hormones varies with nutritional and reproductive status of the animal.

Treatment with long acting slow release preparation of steroids (a combination of 5 mg of oestradiol vaterate – Progynon depot and 50 mg of hydroxy progesterone caproate – Proluton depot) intramuscularly in castor oil will induce oestrus in 7 to 8 days with better conception rate. PMSG can be used in heifers at an age at which it is economically desirable to start a reproductive process. Since there is chance of multiple ovulation, it is better to skip the induced oestrus and breed the animal during the subsequent oestrus.

Functional anoestrus due to ovarian inactivity is a difficult and expensive problem to tackle in buffaloes and heifers. It may be due to an inadequate hypothalamic stimuli for release of gonadotropic hormones. Treatment with synthetic GnRH is one of the choice with significant results (Receptal – 5 ml i/m). Initial use of GnRH (Fertagyl – 2.5 ml i/m) followed by PMSG (Folligon – 500 IU i/m) after 7 days for two consecutive days produce better results. Short term steroid treatment (50 mg of progesterone depot i/m) daily for 5 days followed by 5 mg of oestradiol valerate on 7th day is effective in treating functional anoestrus in cows. Administration of 300 mg of clomiphene citrate as a suspension following the administration of 1% $CuSO_4$ solution (200ml) daily as a drench for five consecutive days results in induction of oestrus.

Postpartum anoestrus is a common reproductive disorder in high producing cattle and buffaloes lead-

ing to prolonged interoestrus interval and thereby reducing the calf crop and milk production. The basic causes are not always apparent and many factors like nutrition, summer stress, gross uterine pathology and managemental practices are implicated in its causation. Since phosphorus deficiency can lead on to anoestrus, supplementation of phosphorus orally and/or parenterally may help in tackling postpartum anoestrus.

Administration of a highly potent analogue of GnRH (Buserelin – 12 μg) after 28 days of parturition had beneficial effect in resuming ovarian activity and oestrus sufficiently earlier, thereby increasing conception rate. Application of a paint consisting of 1% Lugol's iodine solution to the os cervix initiated oestrus behaviour within 5 to 28 days post-treatment. The action of Lugol's iodine in the induction of oestrus was thought to be due to stimulatory effect on hypothalamus or by the release of uterine luteolytic factor acting via the utero-ovarian or utero-pituitary-ovarian pathway.

Induction of fertile oestrus in postpartum anoestrous buffaloes and cattle can be achieved by oral feeding of progesterone for 14 days (melegesterol acetate, MGA-0.5 to 1.0 mg) and a single intramuscular injection of oestrogen (oestradiol benzoate, OB-250 μg), 48 hours after the last day of MGA feeding.

Administration of progesterone for a period of 5 days (50 mg of Duraprogen) consecutively and its subsequent withdrawal create a change in the high progesterone level and initiate follicular development by way of negative feedback mechanism. To improve conception rate in induced oestrus, gonadotropin have been frequently used with progestogens to stimulate follicular growth and ovulation. Crestar with an ear implant containing 3 mg norgestomet and 2 ml Crestar injection containing 3 mg norgestomet and 5 mg oestradiol valerate intramuscularly on the day of implant insertion followed by 500 IU of PMSG on the day of implant removal (10 days after insertion) produce better results.

Suboestrus

Suboestrus condition is common among high producing animals in early postpartum. It is believed that in bovines retention of corpus luteum may occur in connection with pathological condition of uterus. PGF_2a and its analogues have luteolytic activity from day 5 to 16 of oestrous cycle.

A single dose regimen of PGF_2a or its analogues after detecting corpus luteum by per-rectal palpation of ovaries or blind administration of double dose regimen of prostaglandins 10 to 12 days apart can be effectively practised for tackling suboestrus by synchronizing oestrus and fixed time breeding of animals.

Induction and synchronization of oestrus are easily achieved by using PGF_2a or its analogues, but may not induce overt oestrus in all suboestrous cows. On the contrary, intrauterine infusion of Lugol's iodine result in induction and better expression of oestrus, but the synchronization of oestrus will not be precise.

Prostaglandin and its analogues are effective in low doses when infused intrauterine along with dilute solution of Lugol's iodine in suboestrous cows. Here detection of oestrus and fertility are higher compared to Lugol's iodine or prostaglandins alone. Two injections of PGF_2a or its analogues through intravulvo submucosal route (IVSM) at 24 hr interval are more effective than a single injection in luteolysis during early stages of cycle to tackle suboestrus condition.

Animals suffering from Cu deficiency may fail to exhibit oestrus signs. Hence oral administration of 1% $CuSO_4$ solution (30ml daily) will be effective in reducing the incidence of suboestrus due to Cu deficiency.

Delayed ovulation

Usually cows exhibit oestrus for a duration of 18 to 24 hrs and ovulation occur 6 to 12 hours after the end of oestrus. Spermatozoa survive in the female reproductive tract retaining fertilizing ability for a maximum duration of 24 hours and the ovulated oocytes are viable for a maximum duration of 12 hours. Therefore precise synchrony between ovulation and insemination is essential for optimum conception rate. Usually insemination is preferred towards the mid to late stage of oestrus *i.e.*, 12 hrs after the onset of oestrus.

Now a days, there is high incidence of delayed ovulation/prolonged oestrus in our crossbred cattle leading to infertility probably due to asynchrony in ovulation and insemination resulting in either ageing of sperms or ova at the time of fertilization. To overcome this condition, identify such animals by successive rectal palpation to detect the persisting Graafian follicle. In such animals delayed insemination or double insemination at 24 hr interval can be practised. An ovulatory dose of GnRH (Receptal – 2.5 ml i/m) or hCG (Chrulon – 1500 IU i/m) at the time of insemination or 6 hrs before insemination help in timely

ovulation and fertilization. Parenteral administration of small doses of plain progesterone (Uniprogestin – 8 to 10 mg i/m) at the time of AI was also found to be beneficial.

Cystic ovarian degeneration (COD)

Follicular cyst in a condition affecting high producing animals during early lactation which are maintained under high protein diets. Here large cysts will be formed in the ovary from anovulatory enlarged follicles. Animal shows irregular and frequent heat symptoms. Usually multiple cysts are formed and in most cases it may get corrected naturally when there is a reduction in milk production. In some animals, this cyst may persist and cause atrophic changes to the genitalia due to continued stimulation leading to infertility. Sometimes, follicular cyst may get converted to luteal cyst and the animal will go anoestrous. Hormonal therapy is useful in early stages only when there is an active granulosa cell layer. Treatment include perenteral administration of higher doses of hCG (Chorulon – 3000 IU i/m) or GnRH (Receptal – 5 ml i/m). Usually the cysts will rupture, but there is chance of reappearance of cysts in next cycle. So breeding in the next cycle along with an ovulatory dose of hCG or GnRH is preferred. Sometimes, treatment leads to luteinization of cyst resulting in luteal cyst. They can be treated effectively by using prostaglandins. Administration of hCG 7 to 10 days prior to prostaglandin treatment is beneficial in treating luteal cyst since it promotes the proper luteinization of the cyst for the action of prostaglandins.

Uterine infections

Infections of the different parts of reproductive system result in infertility due to embryonic mortality or due to adverse environment. In such animals, there may be irregular oestrous cycle and usually the discharge is cloudy. Different antibiotics, antiseptics, hormonal and non-hormonal preparations are used extensively to eliminate the infection. Natural service with an infected bull, improper timing of AI and improper hygiene during AI are implicated as the major reasons for this condition. Aseptic collection of uterine discharge and cultural studies revealed mostly of *E. coli* infection. In mixed infection *E. coli*, Staphylococci and Streptococci are predominant. The antibiogram of isolates revealed sensitivity towards different antibiotics such as nitrofurazone, chloramphenicol, gentamicin, streptomycin, oxytetracycline and kanamicin.

The efficacy of treatment in counteracting uterine infection depends on many factors. Aminoglycoside group of antibiotics (Gentamicin, Streptomycin, Kenamicin *etc.*) are less effective in postpartum infection due to the anaerobic uterine environment. Penicillinase producing organisms in postpartum uterus deactivate penicillin group of antibiotics. Presence of pus or organic debris inhibits sulp honamides, aminoglycosides and nitrofurazone. Therapeutic levels of the drug are not usually reached in the deeper layers of uterus and other parts of genital tract during early postpartum period due to diminished absorption.

Judicious selection of antibiotic depending on the condition and antibiogram studies has to be performed for optimum results. Hormonal and non-hormonal ecbolics, uterine antiseptics, oestrogens and pros taglandins are helpful in eliminating infection from the uterus. Resumption of the ovarian cycle by any means found to be beneficial in eliminating infection. The indiscriminate and prolonged use of antibiotics in the absence of drug sensitivity studies had contributed to the emergence of resistant strains. Thus isolation and determination of drug sensitivity of the causative agent have become very important for effective therapy of gynaecological infections and to limit the emergence of drug resistant strains.

Repeat breeding

Repeat breeder is an animal with apparently normal genitalia and normal oestrous cycles which fails to conceive even after three or more consecutive inseminations using semen from a fertile male. Detailed examination and time consuming trials are needed to settle such animals. Ovulatory disturbances and embryonic mortality along with managemental factors are contributed to the causation.

It is possible that repeat breeding in otherwise healthy cows may be due to macro mineral and/or trace element imbalance or deficiency. Significant diffe rence existed between repeat breeder and regular breeding cows with regard to serum Cu, Mn, Zn, Ca and P,levels improved after multimineral supple mentation.

Significant improvement in conception rate was observed in buffaloes by showering water or providing wallowing facilities during summer months which may be due to thermolysis resulting in embryonic mortality.

Spontaneously occurring asynchrony between the embryo and maternal environment is a cause of early

embryonic mortality which is the prime cause of repeat breeding. Administration of progesterone depot (Proluton depot – 500 mg) on day 5 of oestrous cycle following AI found to increase the conception rate.

Since luteal insufficiency may be a cause of early embryonic mortality, administration of hCG (Chorulon – 1500 IU i/m) on the 4th or 10th day of insemination found to favour the conception rate.

Mild infections of the uterus can be eliminated by flushing out the uterine horns with normal saline using Foley's catheter was found to improve the conception rate in repeat breeders. Post AI administration of non-spermicidal antibiotic may be useful in counteracting mild uterine infection.

Serum glucose level also plays a significant role in conception. Animals suffering from hypoglycaemia during oestrus records low conception rate. Hence parenteral glucose administration along with A.I. and providing rest to animals, which are taken for a long march to insemination centre, before and after A.I. help in improving conception rate.

Immunoinfertility may be a cause of repeat breeding. Providing sexual rest by skipping 2 or 3 cycles without insemination favour the reduction in antibody response. Insemination using fresh semen to avoid egg yolk components, using washed spermatozoa to avoid seminal plasma antigens *etc.* are also tried. Administration of corticosteroids (*eg.* Dexamethasone – 8mg) along with insemination can also be attempted.

Induction of oestrus during mid luteal phase using prostaglandins and inseminating in the next cycle can also be adopted as a treatment.

Treatment using 25 to 30ml of homologous plasma (plasma collected from same animal and infused i/u) is another method. Administration of 10IU of oxyto cin along with insemination favours the transport of the sperms in the female reproductive tract and hence can be tried in cases with kinked cervix.

A thorough understanding between the vet erinarian and farmer, detailed and follow-up examination of the animal and a perfect recording system of the observations made are very important in the evolution of a rational treatment for repeat breeder animals.

Augmentation of fertility by reducing the intercalving interval and AI index is inevitable for optimum return from dairy industry.

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Transgenic technologies have been applied to express foreign proteins in the mammary gland for the pharmaceutical industry. However, the transgenic technology can also be used to alter the nutritional, antimicrobial and functional properties of milk.

The progress in transgenic technology is much slow because –

(1) Many important economic traits (like milk yield) are controlled by multiple genes.

(2) Technology is new, for e.g.: Elimination of b - lactoglobulin in cattle is needed for decreased allergenic response but this requires genetic engineering of embryonic stem cells which presently is not developed for dairy cattle.

(3) Expensive and risky affair, limits the exploitation for few traits.

(4) Results obtained in mice are not directly applicable to farm animals because expression obtained in transgenic mice is lacking when the same transgene is used in farm animals.

Future Research:

◆ Research on the fundamental chemical properties of milk protein, ways of modification and measurement of its impact on the quality of milk. Altering the characteristics of one component to enhance a particular processing feature may make milk unsuitable for other uses. During the next decade, dairy scientist must determine which approach is most efficacious to achieve the desired goal.

◆ Standardisation of modern technologies to different conditions and their economic feasibility should be evaluated. If modification is by transgenesis, inheritance pattern of transgene in a population should be studied.

◆ It is necessary to think about nature of milk after making changes to the amount or structure of one component. Due to complex interactions, milk may change dramatically. When milch animals are used for synthesis of specific proteins for pharmaceutical or industrial use, this is not likely to be important. But, inconvenience may occur if that milk is used for human consumption.

◆ Well-being of neonates consuming modified milk from their mother should be looked after.

◆ Changes would be required in diet of dairy animals if modifications were brought especially in protein part of milk.

The new technology should be economical, sustainable and should satisfy the ethical norms.