

## Modern Concept in Optimizing Reproductive Efficiency of Seasonal Breeders

Ashish Mishra\*, I.J. Reddy and Sukanta Mondal

National Institute of Animal Nutrition and Physiology, Bangalore-560030, India.

### Abstract

The control and manipulation of reproduction is the subject of scientific interest for many years. High level of reproductive performance can be achieved under optimum managerial conditions because reproduction is directly affected by various managerial factors. Manipulation of these factors causes changes in reproductive performance. Seasonal breeders are animals that successfully mate during certain times of the year. These times of year allow for births with an optimum condition for the survival of the young. Seasonal breeders like sheep and goat are convenient domestic species for biological investigation and application because these species have diversified products of commercial value. To maximize the production potential of seasonal breeders it is essential to increase the reproductive rate. There are number of factors that affect reproductive efficiency such as climate, stress factors other than heat stress, social environment, nutrition, disease, population density, gonadotropins, photoperiod. Globalised research has generated number of important technological reproductive methods that can be applied to increase the reproductive efficiency of seasonal breeders. Most of the recent developments in optimizing reproductive efficiency are expensive but improve reproductive performance.

\*Corresponding Author:

Ashish Mishra

Email: [ashishvet1@gmail.com](mailto:ashishvet1@gmail.com)

Received: 21/09/2015

Revised: 28/09/2015

Accepted: 29/09/2015

**Key words:** Seasonal breeders, Modern concept, Reproductive efficiency.

### 1. Introduction

The control and manipulation of reproduction is the subject of scientific interest for many years. High level of reproductive performance can be achieved under optimum managerial conditions because reproduction is directly affected by various managerial factors. Manipulation of these factors causes changes in reproductive performance. Biotechnology, one of the most fastest growing scientific disciplines of recent progress of science. Recent advances in technology such as nuclear biology, genetic engineering and stem cell research have made scientists to have wide vision on the design and function of living organisms. It is also expected that by the next decade, there may be revolution in commercial livestock production as well as other animal related sectors. Development of novel reproductive biotechnologies, which are also known as assisted reproductive techniques (ARTs) that include artificial insemination (AI), embryo transfer (ET), multiple ovulation embryo transfer (MOET), estrus synchronization and superovulation, laparoscopic ovum pick up (LOPU), in vitro maturation, fertilization and culture (IVMFC), intracytoplasmic sperm injection

(ICSI), cryopreservation of sperm, oocytes and embryos, sperm and embryo sexing, embryo splitting, embryo cloning, nuclear transfer (NT), gene transfer etc. The application of these biotechnologies enables to increase the rate of genetic progress, reproductive efficiency and significant increase in productive ability (Rahman *et al.*, 2008). These ARTs allow animals of high genetic merit to produce more offspring than would be possible by natural breeding. Modern ARTs are being used to improve and preserve livestock genetics as well as enhancement of reproductive efficiency.

Seasonal breeders are animals that successfully mate during certain times of the year. These times of year allow for births with an optimum condition for the survival of the young. Sexual interest in these animals are expressed and accepted during this period only. Female seasonal breeders have one or more estrus cycles in season. But at other times of the year, they are in anestrus. Male seasonal breeders may exhibit changes in testosterone levels, testes weight and fertility depending on the time of year. Seasonal breeders like sheep and goat are convenient domestic species for biological investigation and application

because these species have diversified products of commercial value (Amoah and Gelaye, 1997). Besides, these have a relatively short generation interval as compared to cow, the predominant livestock species. Biotechnological alterations could make significant impact on the quality of sheep and goat production.

## 2. Factors Affecting Reproductive Efficiency

To maximize the production potential of seasonal breeders it is essential to increase the reproductive rate. There are number of factors that affect reproductive efficiency such as climate, stress factors other than heat stress, social environment, nutrition, disease, population density, gonadotropins, photoperiod etc.

### 2.1 Climate

The detrimental effects of high ambient temperature and heat stress on reproductive performance are well known. Impact of temperature is direct as a result of increased body temperature or compensatory changes in blood flow. It may be indirect through hypothalamus involving changes in appetite or feed intake and body metabolism. In females, heat stress during the first week of pregnancy results in higher embryo mortality and subsequent abortions. The impact of extreme climatic condition leads to most of the reproductive problems. The physiological effects of temperature and humidity on reproductive outcome are important to consider. The Temperature-Humidity Index (THI) over the physiologically stressful range (>72 THI) affect thermostability of animals that leads to number of reproductive abnormality. Heat stress alter the normal follicular dynamics pattern. Follicular estradiol levels have shown to be decreased during heat stress, causing disruption of normal folliculogenesis of the first wave dominant follicle and the second wave dominant follicle appears early but function normally. Possibly due to impaired oogenesis, heat stressed dairy animals have less fertile oocytes (Wolfenson *et al.*, 1995). Furthermore, heat stress alter steroid production and metabolism, particularly progesterone is altered. These imbalances affect estrus, embryo survival and follicular development in the ovary. Conceptus weight is also affected during heat stress since feed intake is affected due to high temperature. In Male heat stress cause decrease in sperm motility, increase in sperm abnormalities, decrease sperm output and lower libidos (Wettemann and Bazer, 1985). Heat stress cause increase in intracellular reactive oxygen species (ROS) and decrease in glutathione (GSH) level which is detrimental to embryo survivability. Oxidative stress due to reactive oxygen species (ROS) produced by

cellular metabolism have regulation on various reproductive process like cyclic endometrial and luteal changes, follicular development, ovulation, fertilization, embryogenesis, implantation, placental differentiation and growth. Oxidative stress cause several pregnancy related disorders such as preeclampsia, spontaneous abortion, intrauterine growth retardation leading to pregnancy loss, defective embryogenesis and teratogenicity (Gupta *et al.*, 2007).

### 2.2 Stress Factors Other Than Heat Stress

Any factors affect animal's homeostasis or equilibrium result stress response. These factors may be physical, chemical or emotional. Stress is generally considered to be suppressive to reproductive function. A degree of stress is beneficial for adaptation and survival but chronic or long term stress is detrimental. The impact of stress on the endocrine, immune and nervous systems compromise animal performance that prevents optimum reproduction. Acute stress due to sudden and severe stressor activates the adrenal gland for the quick release of adrenaline and noradrenaline. These hormones cause physiological disturbances that include increases in heart rate, muscle tone and alertness required for animal to flee from danger. These hormones can either stimulate or inhibit contractions and motility of the muscles of the reproductive tract. This, in turn, affects the movement of sperm and eggs for fertilization, the timing of embryos reaching the uterus and the number of embryos that actually survive to implantation. Where as chronic stress cause activation of hypothalamic-pituitary-adrenal axis (HPA) and results increase in release of glucocorticoids, mainly cortisol and corticosterone from the adrenal gland resulting mobilization and redirection of energy reserves through glycogenesis and lipolysis. Continuous activation of the HPA severely affects animal productivity. This chronic stress promotes depression or lethargy, reduced immunity and impaired reproductive function (Varley, 1994).

### 2.3 Social Environment

Social environment include animal-animal and human-animal interactions which have dramatic impact on reproductive efficiency. Social environment is reflected in age at puberty, libido, behaviour at estrus, mating behaviour, estrous cycle, embryo survival and maintenance of pregnancy. Study tells animals reared in groups with others reach puberty sooner, have stronger mating responses and are more interested in partners than are those animals who reared without groups (Hemsworth *et al.*, 1982). Chemical compound pheromones play important role to stimulate animals for these activity. Lack of this stimulus leads to delayed

age of conception, decreased calving rates and smaller litter sizes etc.

#### 2.4 Nutrition

Nutrition influence reproduction in number of ways. Feed quality and composition are not only important which affect reproduction but some substances like mycotoxins found in poor quality feed can adversely affect reproduction directly. Therefore lack of feed ingredients of consistently high quality leads to nutritional deficiencies which cause serious reproductive problems. Studies indicate that as milk yields increase, there is decreases in reproductive efficiency due to partitioning of nutrients due to additional physiological demands of food (Nebel and Jobst, 1998). So proper feeding is important for better reproductive efficiency. There must be provision of properly balanced ration because both excess nutrients and deficient nutrients can cause a problem with reproductive performance. Energy deficiencies can cause number of physiological effects that can severely decrease reproductive efficiency within herds. Energy deficiencies with high levels of protein are thought to cause excess levels of circulating ammonia to flow through the liver, resulting inactive ovaries, endometritis and early embryonic death.

#### 2.5 Disease

Diseases like metritis, mastitis, cystic ovaries, retained placenta influence conception rate. Pregnancy rates are influenced by conception rate and estrus detection efficiency. Estrus detection efficiency also influences conception rate because there should be proper identification of the beginning of estrus to inseminate for maximum fertility which in turn affect reproductive efficiency of animals. It is to be noted that estrus detection accuracy is not the only thing that affects conception rate but physiological stress from increased milk production or heat stress or diseases mentioned above are other factors that can also influence conception rate (Gwazdauskas *et al.*, 1983). Among diseases causing decreases in conception rate, lameness is a major contributor. Lameness also affects calving to conception intervals and services per pregnancy. Services per pregnancy is adversely affected by many factors including decreased estrus detection accuracy, improper A. I. technique, reproductive pathology, lameness and heat stress (Hernandez *et al.*, 2001).

#### 2.6 Population Density

Overcrowding and disruptive social interactions stimulate stress responses that lower reproductive efficiency (Hemsworth *et al.*, 1986). Chronic fighting lowered performance in group. Much of the problem

arises from aggression associated with group feeding. So individual feeding can eliminate that aggression and overcomes the problems.

#### 2.7 Gonadotropins

Hypothalamus is considered to be the central control for reproduction. So hypothalamus is affected by the factors that determine seasonal breeder to be ready for mating. This is achieved specifically through changes in the production of the gonadotropin releasing hormone (GnRH). GnRH acts on pituitary to promote secretion of gonadotropins (LH and FSH). Factors that affect release of GnRH cause disturbance in secretion of gonadotropins which affect cyclic condition of animal.

#### 2.8 Photoperiod

Photoperiod regulate the mating behaviour of a seasonal breeder. Photoperiod likely affects the seasonal breeder through changes in melatonin secretion by the pineal gland that ultimately alters GnRH release from hypothalamus. Day length variations with latitude impact breeding. For example, sheep and goats in tropical climate breed throughout the year while those in temperate climate breed certain time of the year. Females are generally more sensitive to changes in day length. On the basis of day length variations, seasonal breeders can be divided into groups as long day breeders cycle and short day breeders cycle. Long day breeders cycle are those when day light get longer like in spring season and are in anestrus during fall and winter. Example of long day breeder among domestic animal is horse. Where as short day breeders cycle are those when the length of day light shortens like in fall and are in anestrus during spring and summer. Examples of short day breeder among domestic animals are sheep and goat. The decreased light during the fall decreases the activation of retinal nerves, so there is decrease in inhibition of the pineal gland resulting an increase in melatonin because excitation of the superior cervical ganglion decreases. Increase in melatonin results an increase in GnRH that cause increase in LH and FSH, which stimulate cyclicity.

### 3. Modern Techniques to Optimize Reproductive Efficiency

Globalised research has generated number of important technological reproductive methods that can be applied to increase the reproductive efficiency of seasonal breeders like sheep and goats. Most of the recent developments in optimizing reproductive efficiency are expensive but improve reproductive performance. They are listed and discussed in below:

- Manipulation of the Breeding Cycle
- Breeding at Younger Ages
- Increasing Fecundity
- Induction of Parturition
- Pregnancy Testing
- Artificial Insemination
- Embryo Transfer
- In vitro Embryo Production and Transfer
- Laparoscopic Ovum Pick Up
- Intracytoplasmic Sperm Injection
- Cryopreservation of Sperm
- Cryopreservation of Oocytes and Embryos
- Sexing of Sperm and Embryos
- Embryo Splitting
- Cloning and Transgenesis

### 3.1 Manipulation of the Breeding Cycle

Manipulation of breeding cycle is made by manipulating the activity of corpus luteum to synchronize the estrus cycle. Synchronization of oestrus during breeding season and anoestrus are made by stimulating the activity of the corpus luteum that produce progesterone in quantity for about 2 weeks and shutting off production sharply at the end of the oestrus cycle. The technique of vaginal progestagen sponges to control oestrus cycle in the ewe is now well developed in France and this system is spreading in different countries. Synchronization of oestrus during the breeding season is useful for A.I. programs. Uses of progestagen as vaginal pessaries for 14 days, subcutaneous implant of progesterone for 14 days to create an artificial cycle or using of prostaglandin  $F_{2\alpha}$  are the common methods to create synchronization of oestrus (Gordon, 1975).

### 3.2 Breeding at Younger Ages

The age at first parturition mainly depends on the growth rate of the lamb. If the feed availability is short or genetically lamb is of slow growth, the age at the first parturition is delayed. Breeding at younger age have number of benefits such as reducing maintenance costs, shortening the generation interval, increasing the genetic gain and increasing lifetime production.

### 3.3 Increasing Fecundity

It is necessary to increase the fecundity of ewes and does to get more profit. But the genetic approach requires more time to introduce, so physiological methods like the use of exogenous gonadotrophin or the immunization against sexual steroids are interesting alternatives. In developing countries farming is generally carried out in harsh environments and it is obvious that increase in fecundity requires good management and food availability, so the application of

these methods are limited to farms in which these requirements are achieved. Increase in fecundity can be achieved by use of PMSG on day 14 of the oestrus cycle and also by immunization of ewes and does against their own steroid hormones which cause increase in the ovulation rate.

### 3.4 Induction of Parturition

Induction of parturition must be compatible with the survival and the normal development of the kid without deleterious effects on lactation and maternal behaviour. Main objective to use induction of parturition techniques is to make able to reduce lamb mortality by the application of well management techniques. These techniques are used in highly sophisticated intensive farming. This technique is based on the injection of dexamethasone a corticosteroid which mimics the action of the signal normally produced by the fetus to initiate parturition. Other compounds used for this purpose are estrogen and prostaglandin. Close observation of the flocks during lambing can help to reduce the lamb mortality (Bindon, 1982).

### 3.5 Pregnancy Testing

In farms early pregnancy detection is necessary to provide pregnant animals better feeding and attention. It is necessary to determine whether the pregnant animal is carrying multiple fetus or not so that special management can be applied before to eliminate lamb mortality at the time of lambing. Several methods have been developed for early pregnancy detection such as radiographic techniques, progesterone and oestrogen tests, ovine placental lactogen, immunological tests, vaginal biopsy, laparotomy, ultrasonic techniques, rectal-abdominal palpation, manual examinations (Gordon, 1975). From these very few can be used practically and economically at farm level, the most practical one for simple early diagnosis is the detection of the fluid filled uterus by ultrasound.

### 3.6 Artificial Insemination

Artificial insemination (A.I) is of great influence in the improvement of productivity. The advantage of A.I. has to be coupled with an appropriate reproductive rate. That means that the A.I. must not have a detrimental effect on conception and lambing rates. The effort is focused to increase the viability of the semen doses, especially testing different diluents. By increasing the viability of refrigerated and frozen semen A.I in sheep and goat can be greatly improved (Maxwell *et al.*, 1983).

### 3.7 Embryo Transfer

Artificial insemination has an important role in the genetic improvement. Through A.I. selection and genetic improvement through cross breeding can be done gradually and economically. But in certain circumstances, when it is necessary to introduce a new breed or to boost the population of a particular breed, the use of the embryo transfer (ET) can be an interesting alternative. ET requires superovulation of donors, breeding of the donors, recovery of eggs, evaluation of the embryos, handling and preservation of embryos, synchronization of recipients, transfer of the embryos. Each of these phases has its own characteristics and problems. Researchers have focused to solve the difficulties in each step and made a viable economical and practical methodology.

### 3.8 In Vitro Embryo Production and Transfer

Embryo production by in vitro includes *in vitro* maturation, fertilization and culture (IVMFC) of embryos, have the potential to improve the number of offspring produced by genetically valuable female. In this technique, immature oocytes are collected from slaughterhouse ovaries by aspiration of follicles or slicing. The various steps involved in IVMFC are in vitro maturation of the immature oocytes, fertilization of the matured metaphase II (MII) oocytes with in vitro capacitated fresh or frozen-thawed semen and culture in vitro of presumptive zygotes for 7-8 days until formation of blastocysts that can be transferred to recipients or cryopreserved for future use (Mishra *et al.*, 2010).

### 3.9 Laparoscopic Ovum Pick Up

Laparoscopic ovum pick up (LOPU) is one of the best techniques for oocyte recovery from live animals. This is the most effective and non invasive technique, which cause less adhesion related problems compared to laparotomy or surgical oocyte collection. Thus LOPU offers the possibility of repeated ovum pick up and allows for repeated production of oocytes/embryos from a single donor. LOPU technique improves poor results observed with superovulation, such as poor ovulation rate, early regression of corpora lutea and poor fertilization (Baldassarre and Karatzas, 2004).

### 3.10 Intracytoplasmic Sperm Injection

Intracytoplasmic sperm injection is the mechanical insertion of a single sperm directly into the ooplasm of a matured oocyte using a microscopic needle. It is a special type of in vitro fertilization (IVF) and one of the advanced ART that is now widely used to overcome male factor infertility in human. In animals including livestock, by applying this technique many live births have been achieved in a number of

species. Although the technique has potential to improve livestock production, however, till now it has not been commercially used in animal production (Jimenez-Macedo *et al.*, 2007).

### 3.11 Cryopreservation of Sperm

Cryopreservation of sperm is an important concept for the advancement of animal production. By this technique sperm can be stored indefinitely, used widely and exported easily. The frozen sperm facilitates exchange of genetic material, allows AI in both the reproductive and non-reproductive seasons and extends the effective reproductive life of a valuable male beyond its own life (Abdullah *et al.*, 2001). Cryopreservation of sperm is a complex process, which involves balancing many factors to obtain satisfactory cryo survival. Ability to cryopreserve sperm of all of the domestic animals is challenging because sperm of different animals are not of similar size, shape and lipid composition, all of which affect cryosurvival. Thus, cryopreservation protocol optimized for sperm of one species may not be ideal for another species.

### 3.12 Cryopreservation of Oocytes and Embryos

Cryopreservation of oocytes and embryos is also another important concept like cryopreservation of sperm for the advancement of animal production. Oocytes or embryos can be stored for a long period of time in liquid nitrogen by using a programmable freezer, quick freezing, direct plunging and vitrification methods. Various cryoprotectants such as glycerol, DMSO, ethylene glycol, polyethylene glycol and sucrose can be used to preserve oocytes and embryos. Among all these cryoprotectants, ethylene glycol is considered to be the most suitable cryoprotectant though excellent results are also reported with other cryoprotectants like DMSO or glycerol (Fieni *et al.*, 1995; Sharma *et al.*, 2010). Now a days vitrification (solidification of a liquid without crystallization or ice formation, by an extreme elevation in viscosity during cooling) has been adapted for many species to preserve oocytes and embryos which is acting as a noble tool to eliminate freezing damage

### 3.13 Sexing of Sperm and Embryos

Sex predetermination in livestock offspring is now of great demand to provide the most efficient production. Sperm sexing is based on the well known difference in X and Y sperm in the amount of DNA present (Johnson, 2000). Though use of sex-sorted sperm for AI, IVF or ICSI has been promoted as a means of increasing the efficiency of reproduction in animals but the process of commercialization of sexed sperm has limitation of high costs, complexity of

implementation and lower pregnancy rates (Seidel, 2007). Sexing of embryos in domestic species is by the application of Polymerase Chain Reaction (PCR) technique to amplify sex-specific genes, followed by electrophoresis. But the economic factor should be considered in sexing of sperm and embryos.

### 3.14 Embryo Splitting

The facility to split embryos generating identical twins was developed few years ago. The purpose of embryo splitting is to provide genetically identical twins to be used for experimental purposes or to increase the number of transferable embryos (Holtz, 2005). Few studies have been reported on embryo splitting in animals. In a study of goat splitted embryo transfer, 59% of the embryos were carried to term. However, only 9% kidding rate was found when used cryopreserved splitted embryos. In a very interesting study, splitted early embryos when transferred to genetically identical females, there was genetically an identical twin from these foster females (Oppenheim *et al.*, 2000).

### 3.15 Cloning and Transgenesis

Cloning is the asexual production of genetically identical animal that can be obtained by embryo splitting or by nuclear transfer (NT). Though cloning can be generated by embryo splitting, however embryo sectioning for more than once will drastically reduce chances of survival (Holtz, 2005). Therefore NT can be used to generate larger clones. The NT involves the transfer of nuclei from fetal or adult cells into

enucleated oocytes matured in vivo or in vitro. For this purpose fetal fibroblast cell or a variety of adult cells including mammary epithelial cells, granulosa cells, sertoli cells and skin cells can be used. Birth of Dolly who had been cloned by transfer of adult somatic cell to an enucleated oocyte (Wilmut *et al.*, 1997). Cloning and NT technology benefit the propagation of genetically superior individuals. Transgenesis or gene transfer has the potential to play an important role in accelerating and facilitating genetic improvement. A transgenic animal may be defined as one containing recombinant DNA molecules in its genome that were introduced by intentional human intervention (Wall, 1996). Transgenic animals can be produced by several techniques, including DNA transfer by retroviruses, microinjection of genes into pronuclei of fertilized ova, injection of embryonic stem cell or germ cells into the blastocyst. However, the most common method for the generation of transgenic animals is the microinjection of DNA into the pronuclei of zygotes (Baldassarre *et al.*, 2003).

## 4. Conclusion

Animal production directly depends on reproduction and optimum reproductive efficiency is prerequisite for animal production. Seasonal breeders like sheep and goat have diversified products of commercial value. To maximize the, reproductive efficiency and significant increase in productive ability of seasonal breeders number of biotechnological reproductive methods discussed above can be applied.

## References

- Abdullah RB, Kanwal KDS and Wan Khadijah WE (2001). Advancement of animal reproductive biotechnology in South East Asia. *Asian Australasian Journal of Animal Science*, 14: 61-71.
- Amoah EA and Gelaye S (1997). Biotechnological advances in goat reproduction. *Journal of Animal Science*, 75: 578-585.
- Baldassarre H and Karatzas CN (2004). Advanced assisted reproduction technologies (ART) in goats. *Animal Reproduction Science*, 82-83: 255-266.
- Baldassarre H, Keefer C, Wang B, Lazaris A and Karatzas C N (2003). Nuclear transfer in goats using *in vitro* matured oocytes recovered by laparoscopic ovum pick up. *Cloning Stem Cells*, 5: 279-285.
- Bindon BM (1982). Techniques for control of reproduction in sheep Address to Australian Poel Dorset Association Field Day "Spring Farm" - Armidale Australia - 23rd July 1982.
- Fieni F, Beckers JP, Buggin M, Bruyas JF, Perrin J, Daubie M and Tainturier D (1995). Evaluation of cryopreservation techniques for goat embryos. *Reproduction Nutrition Development*, 35: 367-373.
- Gordon I (1975). The use of progestagens in sheep bred by natural and artificial insemination. *Annals of Biology, Animals Biochemistry and Biophysics*, 15 (2): 303-315.
- Gupta S, Agarwal A, Banerjee J and Alvarez J G (2007). The role of oxidative stress in spontaneous abortion and recurrent pregnancy loss: a systematic review. *Obstetric Gynecological Survey*, 62(5): 335-347.
- Gwazdauskas FC, Lineweaver JA and McGilliard ML (1983). Environmental and management factors affecting estrous activity in dairy cattle. *Journal of Dairy Science*, 66: 1510-1514.
- Hemsworth PH, Barnett JL and Hansen C (1986). The influence of handling by humans on the behaviour reproduction and corticosteroids of male and female pigs. *Applied Animal Behaviour Science*, 15: 303-314.
- Hemsworth PH, Cronin GM and Hansen C (1982). The influence of social restriction during rearing on the sexual behaviour of the gilt. *Animal Production*, 35: 35-40.
- Hernandez J, Shearer JK and Webb DW (2001). Effect of lameness on the calving-to-conception interval in dairy cows. *Journal of American Veterinary Medical Association*, 218(10): 1611-1614.

- Holtz W (2005). Recent developments in assisted reproduction in goats. *Small Ruminant Research*, 60: 95-110.
- Jimenez-Macedo AR, Paramio MT, Anguita B, Morato R, Romaguera R, Mogas T and Izquierdo D (2007). Effect of ICSI and embryo biopsy on embryo development and apoptosis according to oocyte diameter in prepubertal goats. *Theriogenology*, 67: 1399-1408.
- Johnson LA (2000). Sexing mammalian sperm for production of offspring: The state-of-the-art. *Animal Reproduction Science*, 60-61: 93-107.
- Maxwell WMC, Butler LG and Wilson HR (1983). Proc of the fifteenth Ann Conf Aust Soc for Reproductive Biology Canberra Australia Sept 4-7 1983.
- Mishra A, Chandra V and Sharma GT (2010). Effect of epidermal growth factor on *in vitro* maturation of buffalo oocytes and embryo development with insulin like growth factor-1 and  $\beta$ -mercaptoethanol. *Indian Journal of Animal Science*, 80(8): 721-724.
- Nebel RL and Jobst SM (1998). Evaluation of systematic breeding programs of lactating dairy cows: a review. *Journal of Dairy Science*, 81: 1169-1174.
- Oppenheim SM, Moyer AL, Bondurant RH, Rowe JD and Anderson GB (2000). Successful pregnancy in goats carrying their genetically identical conceptus. *Theriogenology*, 54: 629-639.
- Rahman ANA, Abdullah R B and Khadijah WEW (2008). A review of reproductive biotechnologies and their application in goat. *Biotechnology*, 7: 371-384.
- Seidel GE (2007). Overview of sexing sperm. *Theriogenology*, 68: 443-446.
- Sharma GT, Dubey PK and Vikash C (2010). Morphological changes DNA damage and developmental competence of *in vitro* matured vitrified-thawed buffalo (*bubalus bubalis*) oocytes: A comparative study of two cryoprotectants and two cryodevices. *Cryobiology*, 60: 315-321.
- Varley MA (1994). Stress and reproduction in the pig In: Livestock Production for the 21st Century Priorities and Research Needs PA Thacker (Editor) University of Saskatchewan Saskatoon Saskatchewan Canada pp 147-157.
- Wall RJ (1996). Transgenic livestock: Progress and prospects for the future *Theriogenology*, 45: 57-68.
- Wettemann RP and Bazer FW (1985). Influence of environmental temperature on prolificacy of pigs. *Journal of Reproduction and Fertility Supplement*, 33: 199-208.
- Wilmut I, Schnieke AE, McWhir J, Kind KL and Campbell KHS (1997). Viable offspring derived from fetal and adult mammalian cells. *Nature*, 385: 810-813.
- Wolfenson D, Thatcher WW, Badinga L, Savio JD, Meidan R, Lew BJ, Braw-Tal R and Berman A (1995). Effect of heat stress on follicular development during the estrous cycle in lactating dairy cattle. *Biology of Reproduction*, 52: 106-113.