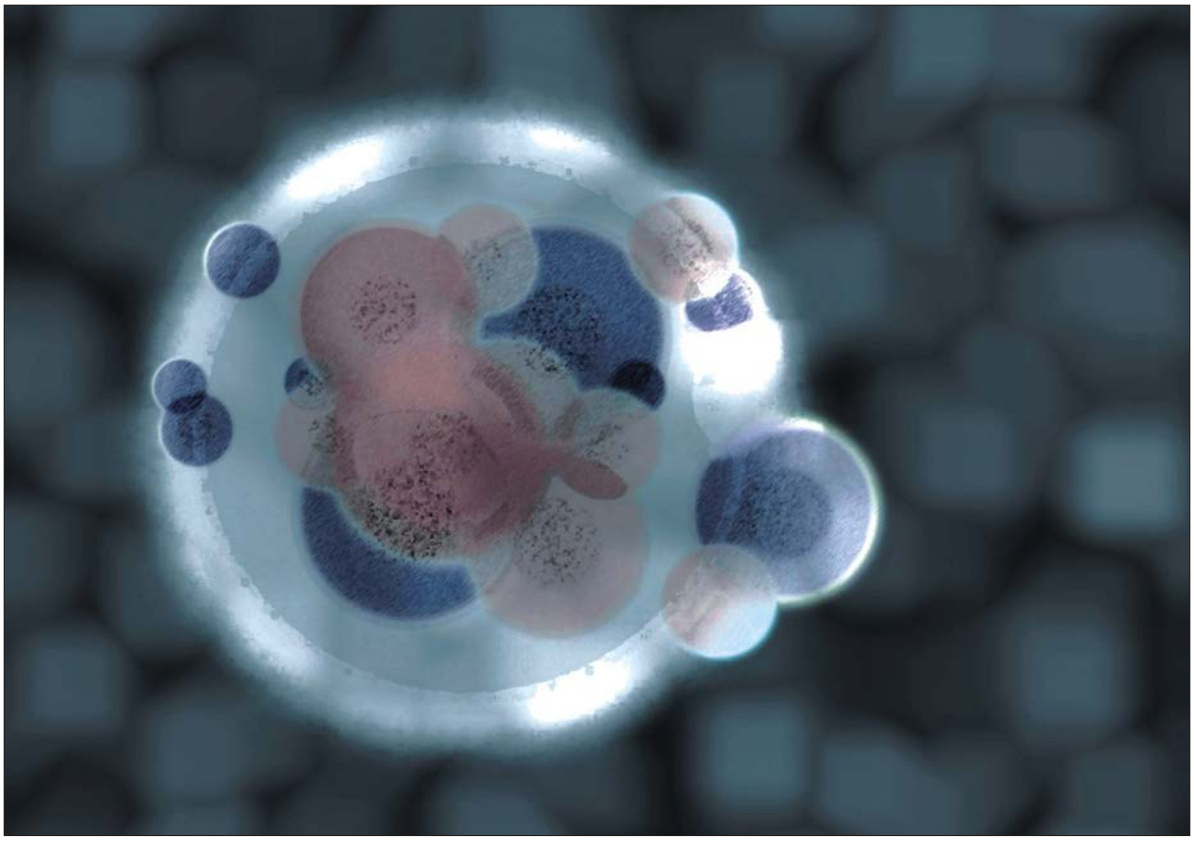

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Reproductive Technology in Farm Animals: New Facets and Findings: A Review

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Abstract: Farm animal selection and reproduction are on the threshold of the application of new biotechnologies. Modern biotechnologies will allow advances to be made. Research into physiology and embryology has provided a basis for the development of technologies that increase productivity of farm animals through enhanced control of reproductive function. The livestock provides many opportunities to utilize these disciplines and evolving competencies. Artificial insemination, embryo transfer, *in vitro* fertilization, cloning, transgenics and genomics all are components of the tool box for present and future applications. Understanding the mechanisms that regulate reproductive function has important implications for this diverse field. Several peptides play a role in determining the normal functioning of the neuroendocrine regulation of reproduction. Kiss1 neurons have emerged as primary transducers of internal and environmental cues to regulate the neuroendocrine reproductive axis. Leptin serves as a metabolic signal that acts on the hypothalamic-pituitary-ovarian axis to enhance GnRH and LH secretion and ovarian function. Leptin effects on Gonadotropin-Releasing Hormone (GnRH) /LH secretion are mediated by NPY and kisspeptin. In recent years, livestock productivity has been increased by improved reproduction. Various techniques have been developed and refined to obtain a large number of offspring from genetically superior animals or obtain offspring from infertile animals. These techniques include: artificial insemination, cryopreservation of gametes or embryos, induction of multiple ovulations, embryo transfer, *in vitro* fertilization, sex determination of sperm or embryos, nuclear transfer, cloning, etc. Further the wide development radio-immuno-assay technology offers wide scope for improving the reproductive efficiency of farm animals. RIA technique for early non-pregnancy diagnosis can be integrated in to AI programmes in order to increase their effectiveness, reduce the unproductive period of dairy cows and increase the economic benefits to farmers. The greater challenge lies ahead for animal researchers is to integrate and potentially exploit these novel technologies in a society-friendly manner. Accepting this challenge and working towards achieving such targets should enable us to reap maximum benefits from the farm animal sector.

Key words: Cloning, transgenics, nuclear transfer, kisspeptin, RIA, leptin, gene transfer

INTRODUCTION

Remarkable improvements in livestock productivity in the developed countries have achieved through research into how animals grow and how yields can be influenced and protected, followed by the widespread uptake of new techniques and materials. Many of the advances in improving the feeding, fertility and health of livestock have been possible with use of nuclear techniques (Naqvi *et al*., 2002; Khanal and Munankarmy, 2009). Farm animal selection and reproduction are on the threshold of the application of new biotechnologies. Modern biotechnologies will allow advances to be made. Globally, there is active competition for new technologies that may directly or indirectly affect the future of animal production, including breeding. The combined advances in genetics, embryology and stem cell research open the prospect that high-added value products for agricultural, medical and technical applications will soon become a reality (Srivastava and Sejian, 2010; Meenambigai and Sejian, 2011).

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Reproductive technology encompasses all current and anticipated uses of technology in human and animal reproduction, including assisted reproductive technology, contraception and others (Mapletoft and Hasler, 2005). Research into physiology and embryology has provided a basis for the development of technologies that increase productivity of farm animals through enhanced control of reproductive function. Animal Biotechnology represents an expanding collection of rapidly developing disciplines in science and information technologies. The livestock provides many opportunities to utilize these disciplines and evolving competencies. Artificial insemination, embryo transfer, *in vitro* fertilization, cloning, transgenics and genomics all are components of the tool box for present and future applications (Betteridge, 2003). Individually, these are powerful tools capable of providing significant improvements in productivity. Combinations of these technologies coupled with information systems and data analysis will provide even more significant changes in the next decade. Various techniques have been developed and refined to obtain a large number of offspring from genetically superior animals or obtain offspring from infertile (or sub fertile) animals (Naqvi *et al*., 2001; Blackburn, 2004). To take full advantage of the benefits of assisted reproductive technologies, one must understand the basic physiology of the female and male reproductive systems as well as various methods to synchronize reproductive cycles (Paterson *et al*., 2003).

Based on the progress in scientific knowledge of endocrinology, reproductive physiology, cell biology and embryology during the last fifty years new biotechniques have been developed for and introduced into animal breeding and husbandry (Wrathall *et al*., 2004). Among them are oestrus synchronization/induction, artificial insemination, Multiple Ovulation Induction and Embryo Transfer (MOET), *in vitro* embryo production (IVP) and cloning by Nuclear Transfer (NT). The aims of these reproductive technologies were initially to speed up the genetic improvements of farm animals by the increase of offspring of selected males and females and the reduction of the generation intervals. The technique of cloning by nuclear transfer is mainly applied for experimental purposes, with the prospect of a more practical implementation in the near future, with the aims of the enhancement of the uniformity of herds for an easier management or for the multiplication of transgenic animals after gene-targeting (Polejaeva and Campbell, 2000; Meenambigai *et al*., 2009).

Techniques are now available to get genetically improved farm animals in large numbers. This involves collection of the fertilized egg from genetically superior female which ovulate spontaneously or are induced to superovulate (Wolf *et al*., 2000). The above goal can also be achieved by transferring the embryo of genetically improved variety into genetically less desirable females, thus getting many more progeny of superior individuals. The current efficiency for producing transgenic animals particularly farm animals, is low and the cost is high. Success in the production of transgenic farm animals requires an adequate animal facility and dedicated teams of embryologists, veterinarians, animal scientists and molecular biologists. Improvement on the success rate of the transgenic technology largely relies on the application of (1) various classical reproductive and embryological technologies such as artificial insemination, superovulation and reproductive management etc. and (2) newly emerged contemporal technologies such cloning and other assisted reproductive technologies.

NEUROENDOCRINE REGULATION OF REPRODUCTION

Understanding the mechanisms that regulate reproductive function has important implications for diverse field. The neuroendocrine pathway regulating reproductive status is the Hypothalamo-Pituitary-Gonadal (HPG) axis. Reproductive activity is induced by the release of hypothalamic gonadotropin-releasing hormone (GnRH) and the subsequent release of pituitary gonadotropins, Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH). LH and FSH act on the gonads to induce the production of sex steroids and gamete development. Sex steroids, in turn, act on the brain to promote appropriate sexual behaviour. Figure 1-4 describes the neuroendocrine regulation of reproduction in male and female animals. Excitatory amino acids (ExAA) such as glutamate and aspartate are important neurotransmitters that play an important role in neuroendocrine control of anterior pituitary hormone secretion. The gonadotropins LH and FSH control testicular function, including testosterone secretion and spermatogenesis. In male domestic animal species, ExAA agonists, such as n-methyl-D, L-aspartate (NMA), effectively stimulate LH secretion and subsequent testosterone release, by acting within the brain to stimulate secretion of GnRH. Stimulatory effects of ExAA agonists on FSH secretion, however, have not been demonstrated.

Inhibins and oestradiol act directly at the anterior pituitary to decrease expression of the gene encoding the FSH subunit. They act to reduce both transcription and stability of mRNA, effects which override GnRH action on FSH release. Oestradiol causes a major decrease in FSH,

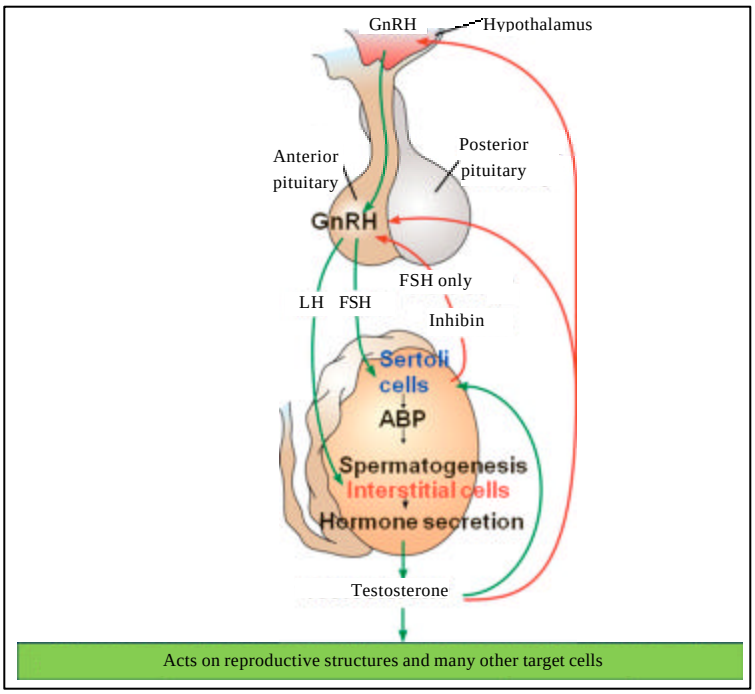

Fig. 1: Overview of reproductive hormone actions in male and the negative feedback systems regulating hormone concentration

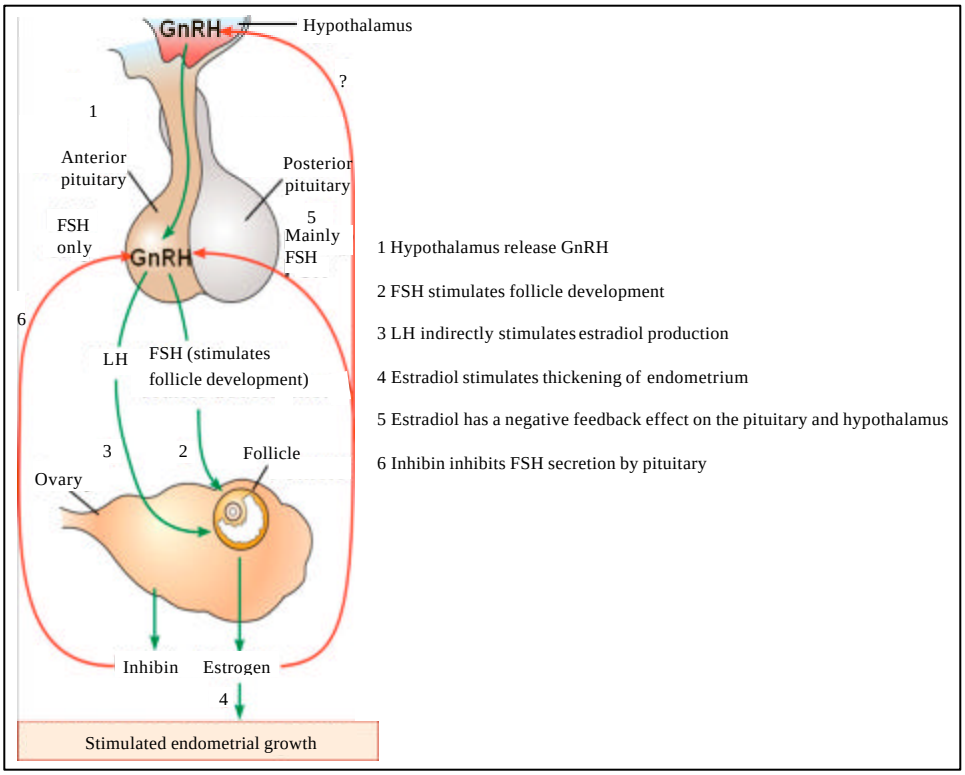

Fig. 2: Role of different reproductive hormones in females during preovulatory phase

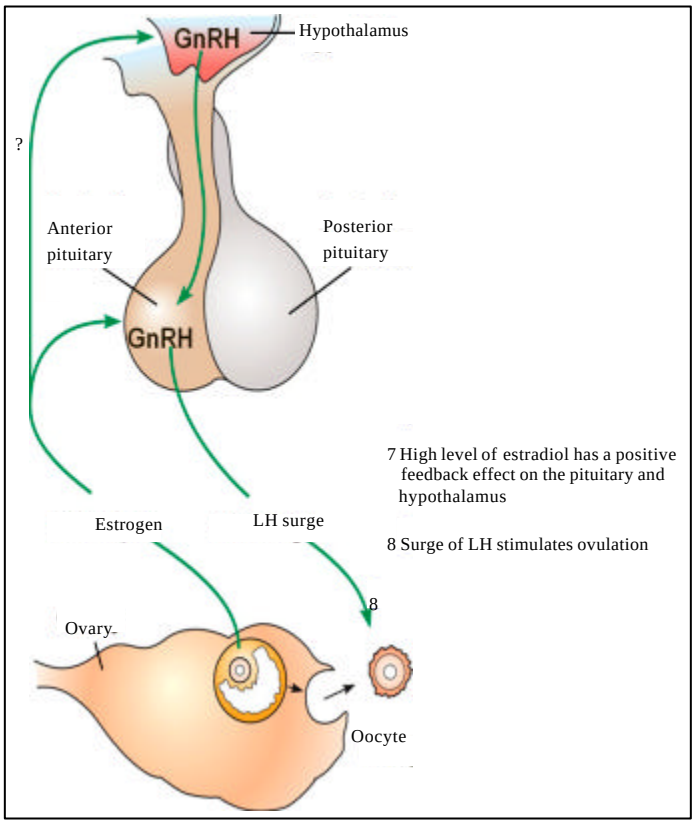

Fig. 3: Role of different reproductive hormones in females during Late preovulatory phase

while LH is initially decreased and then increased. Inhibins suppress FSH, without affecting LH (Mather *et al*., 1992). The initial stages of folliculogenesis occur independently of gonadotrophic hormones. GnRH binds to specific receptors on gonadotrophs and triggers the release of intracellular Ca^{2+}, causing a transient release of both FSH and LH. FSH is the key hormone stimulating the emergence of waves of follicles and its decline is associated with selection of a dominant follicle, which then becomes dependent on LH for its final fate when concentrations of FSH are minimal (Roche, 1996). During the oestrous cycle of ewes, the patterns of secretion of LH and FSH are different, with an increase in LH pulse frequency of 1 per hour during pro-oestrus, while there is a coincident decrease in FSH due to the increase in oestradiol and perhaps inhibins. There is a major depletion of LH after the preovulatory surge. Thus, LH release is a regulated pathway mediated by GnRH action, whereas FSH release is a more constitutive pathway, in which synthesis is followed by release rather than by storage.

Neurons that produce gonadotropin-releasing hormone(GnRH) reside in the basal forebrain and drive reproductive function in mammals. Kisspeptin, the peptide encoded by the KiSS-1 gene, plays an important role in the development and upregulation of the reproductive system (Funes *et al*., 2003; Seminara *et al*., 2003; Greives *et al*., 2008), whereas GnIH has been reported to play an important role in regulating reproduction by downregulating release of pituitary gonadotropins via actions on the HPG axis. Kisspeptin neurons involved in control of reproduction are situated in anteroventral periventricular (AVPV) and arcuate (ARC) nuclei of the brain. The receptor for kisspeptin, G-proteincoupled receptor 54 (GPR54), has been localized to a majority of GnRH neurons (Quaynor *et al*., 2007). It has been established that kisspeptin produces its effects via actions on GnRH neurons (Smith and Clarke, 2007). Figure 5 depicts the hypothetical model of kisspeptin and GnIH action in controlling reproduction in animals.

The expression of Kiss1 in the anteroventral periventricular nucleus (AVPV) is sexually dimorphic and Kiss1 neurons in the AVPV may participate in the generation of the preovulatory GnRH/luteinizing hormone (LH) surge in the female rodent. Kiss1 neurons have emerged as primary transducers of internal and

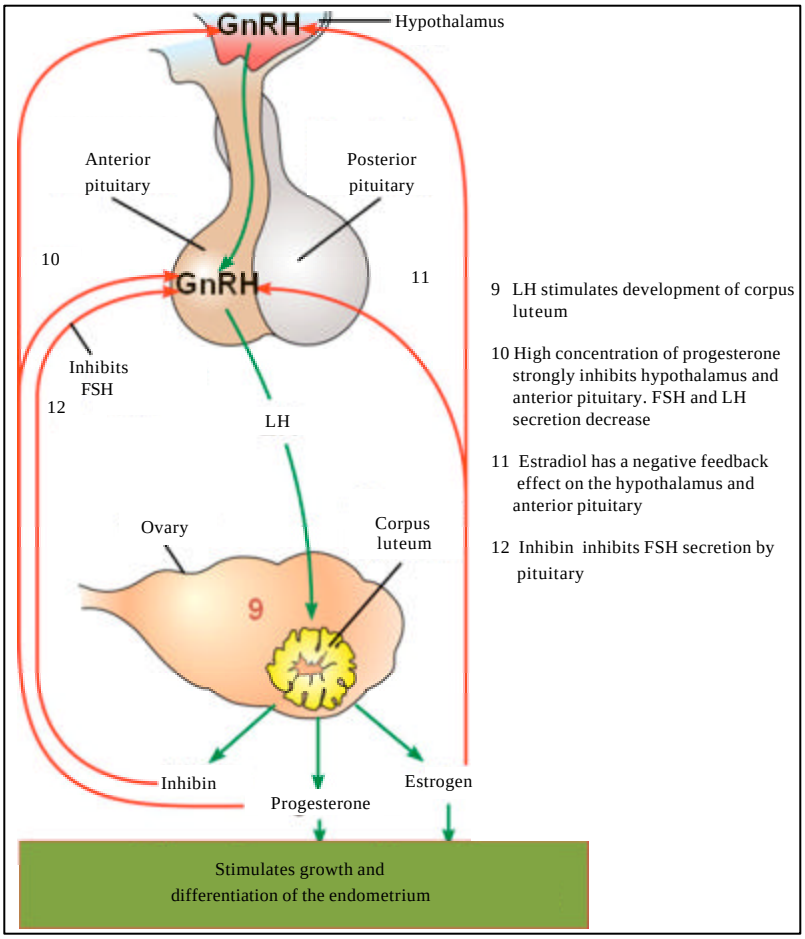

Fig. 4: Role of different reproductive hormones in females during postovulatory phase

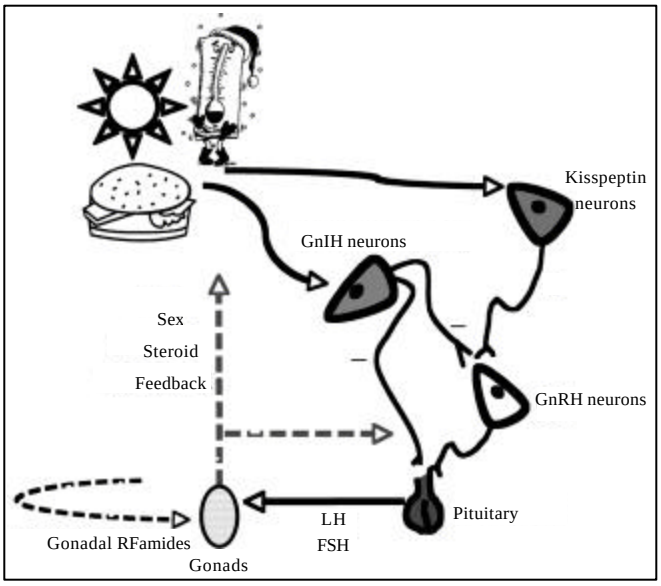

Fig. 5: Hypothetical model of kisspeptin and GnIH action. (Greives *et al*., 2008)

environmental cues to regulate the neuroendocrine reproductive axis (Popa *et al*., 2008). Leptin serves as a metabolic signal that acts on the hypothalamic-pituitary-ovarian axis to enhance GnRH and LH secretion and ovarian function. It has been reported in several farm animals that leptin stimulates steroidogenesis and modulated follicular development (Zachow *et al*., 1999; Agarwal *et al*., 1999; Brannian *et al*., 1999; Campbell *et al*., 2000). Leptin effects on gonadotropin-releasing hormone (GnRH)/LH secretion are mediated by NPY and kisspeptin. Thus, leptin appears to be an important link between metabolic status, the neuroendocrine axis and subsequent fertility in farm animals (Barb *et al*., 2006).

Seasonal breeding in sheep and horse is governed by photoperiod. The signal requires a neuroendocrine transduction (Parvizi, 2000). Concerning photoperiod, dopamine, opioids and melatonin are possibly the best-known mediators between brain and gonadal function (Gallegos-Sanchez *et al*., 1998). The primary site of melatonin reproductive action is on hypothalamus leading to alterations GnRH and thereby altering the LH and FSH secretion. In the pituitary pars tuberalis seems to be the important site of melatonin action to inhibit LH and FSH secretions (Pang *et al*., 1998). There is substantial evidence to support a direct action of melatonin on the gonads. Melatonin modulates the morphology, steroidogenesis or cGMP production of testicular tissues *in vitro* and the Leydig cell is considered by many to be the target site of melatonin action. Similarly, melatonin has been shown to increase progesterone production by corpus luteum or granulosa cells (Baratta and Tamanini, 1992).

Reproductive status is regulated by nutritional feedback to the hypothalamus that controls GnRH and hence LH pulsatile output (Miller *et al*., 2007). Both leptin and insulin have been shown to be involved in the long-and short-term control of reproductive neuroendocrine function in several species, including sheep. Both insulin and leptin stimulates LH secretion (Cunningham *et al*., 1999; Adam *et al*., 2003; Miller *et al*., 2007). Miller *et al*. (2007) further hypothesized that GnRH/LH stimulation by increasing nutritional status is mediated by increased amounts of circulating leptin and insulin entering the brain, down-regulating hypothalamic expression of neuropeptide Y (NPY) and agouti-related peptide (AgRP) and up-regulating expression of proopiomelanocortin (POMC) and amphetamine-regulated transcript (CART). whereas GnRH/LH inhibition by decreasing nutritional status is mediated by decreased amounts of circulating leptin and insulin entering the brain, up-regulating hypothalamic NPY and AgRP expression and down-regulating POMC and CART expression. The GnRH/LH response to an increasing plane of nutrition appears to be mediated by changes in circulating insulin, which enters the hypothalamic CSF and stimulates reproductive neuroendocrine output by inhibiting NPY expression. The GnRH/LH response to a decreasing plane of nutrition appears to be mediated by changes in leptin signaling via increased leptin receptor expression, which inhibits reproductive neuroendocrine output by inhibiting melanocortin activity (Miller *et al*., 2007).

Another important neuroendocrine pathway is composed by opioids. The opioid associated with reproduction are b-endorphin, Met-and leu-enkephalin, Dynorphin A, B and A_1-8. a and b neoendorphin, Nociceptin and Endomorphin-1 and-2 (Dyer *et al*., 1991; Chieng and Williams, 1998; Parvizi, 2000). Genes encoding the three well-known classical opioid receptors are already cloned. The Mor-1, Dor-1 and Kor-1 genes give rise to m-, d-and k-opioid receptors, respectively and possibly also to their subtypes. However the clear role of these opioids and their possible mode of action in farm animals is yet to established. But, it is well known that they strongly modulate the gonadotropin and neurohypophyseal hormone secretion in different reproductive phases. Synthesis of data in Table 1 describes the various peptides and their possible role in reproduction.

FARM ANIMAL CLONING

General description of cloning technology: Clone is an organism or animal that is having the genetic material identical to the ancestor from which its genome has descended. The term cloning is the technique applied to produce identical organisms or animals that have similar genetic material. It can be achieved by two ways. First is by splitting the embryo at an early (pluripotent) stage so that identical twins will be produced. This technique has been performed first in sea urchins, followed by salamanders and farm animals (Vajta and Gjerris, 2006). Initially embryo splitting gained much of importance but later lost the track as it is technically complicated and less efficient because one ovum can give rise to only two identical individuals. Second method is by somatic cell nuclear transfer. The concept of cloning an animal by transfer of somatic cell nucleus was proposed in 1938. Slowly this has been tested and proved to be feasible first in amphibians followed by the demonstration in mice. This technique gained much focus in 1997 when (Wilmut *et al*., 1997) cloned the first sheep Dolly portraying this as an efficient method for farm animal production. In this technique the somatic cell nucleus is transferred into the enucleated cytoplasm of an oocyte. After the transfer of

Table 1: Various peptides involving in reproduction and their possible mechanism of action

Name of the peptides	Possible mechanism of action	References
Placental lactogen	Stimulates mammary growth and milk secretion.	Byatt *et al.* (1992)
Endogenous opioid peptides	Modulation of gonadotrophin secretion via interactions with other neural pathways	Cosgrove *et al.* (1993)
Inhibin (Folliculostatin)	Regulates release of FSH from anterior pituitary	Taya *et al.* (1996)
β-endorphin	Role in modulating GnRH release from the median eminence	Ziecik *et al.* (1999)
Relaxin	Expansion of pelvis	
Dilation of cervix	Ohleth *et al.* (1999)	
Vasoactive intestinal peptide	Role in modulating GnRH release from the median eminence	Ziecik *et al.* (1999)
Neuropeptide Y	Role in modulating GnRH release from the median eminence	Ziecik *et al.* (1999)
Galanin	Role in modulating GnRH release from the median eminence	Ziecik *et al.* (1999)
Calcitonin gene-related peptide	Play a role in regulating blood flow to the uterus during pregnancy and therefore, in fetal growth and survival	Gangula *et al.* (2003)
C-type natriuretic peptide	In female controls embryonic and fetal development while in males controls endocrine function of the testis and the regulation of penile erection	Walther and Stepan (2004)
Leptin	Regulation of gonadotropin secretion in farm animals	Barb and Kraeling (2004)
GnIH	Able to alter pituitary gonadotropin release directly	Osugi *et al.* (2004)
VGF peptide	Neuroendocrine regulation of homeostasis and reproduction	Brancia *et al.* (2005)
Chromogranin-A	Involved in the regulation of spermatogenesis	Payan-Carreira *et al.* (2006)
KiSS-1 peptide	Induces release of LH by a direct effect on the hypothalamus	Arreguin-Arevalo *et al.* (2007)
Neuromedin U	Predominant stimulatory role of in control of the female gonadotropic axis	Vigo *et al.* (2007)
Insulin-like growth factors IGF-I and IGF-II	Stimulates steroidogenesis, stimulates mammary growth and fetal development	Velazquez *et al.* (2008)
Ghrelin	Participate in the regulation of different aspects of the female and male reproductive functions from germ cell production to embryo development	Dupont *et al.* (2010)
Gonadotropic releasing hormone (GnRH)	Stimulates release of FSH and LH from anterior pituitary	Jadav *et al.* (2010)
Terminal ampullae peptide	Participates in regulating sperm proteolytic activity, and performs a crucial role in sperm maturation and degradation of the vitelline coat during fertilization	Ma *et al.* (2010)

nucleus to the enucleated ovum, the ovum is transferred to the womb of a surrogate mother to activate the embryo to start dividing. The technique has been tested in cattle, rabbits, cats and pigs and was found to be successful (Sandoe, 2005). The organisms/animals produced by this technique carry a recombinant gene that is artificially added to the host's original genome in order to direct the expression of a particular protein of interest or alter certain mechanisms of the host.

Pros and cons of cloning technology: Animal cloning ensures the sustainability of the desirable phenotype allowing them for better production of high quality and safe food products. At present the main reason to clone farm animals is to preserve the breeding capacity of genetically elite animals (proven through progeny testing), particularly males and to insure against loss of valuable genetic and characteristic features. Pig cloning involves the use of valuable boars for two reasons. First, because billions of sperm are required for artificial insemination and so any given boar can serve only a small number of females compared with bulls. Second, the most effective way to evaluate the genetic quality of a pig is through a detailed analysis of the carcass. Cloning offers the opportunity to evaluate the quality of a boar by slaughtering it and then using numerous copies of those individuals whose carcasses meet the required standards. In females similar reasons can be given and so oocytes can also be valuable. For other animals it may be to restore their breeding capacity e.g. after injury, disease, old age, or that they have been previously castrated (horses, dogs, cats) (Blasco, 2008; Montaldo, 2006).

Employing this technique, animals mimicking human diseases can be cloned and studied in a better way which would be directly advantageous for human welfare. Cloning is being increasingly used in developmental biology; in the aetiology and pathophysiology of disease; in biotechnology for the production of therapeutic agents e.g., pharmaceutical proteins, organ development, in xenotransplantation and possibly in safety testing (Chaucer, 2003).

Cloning can bring a revolution in the meat industry as the necessity and cost involved in rearing animals is cut off. Cloning could be useful in preserving the endangered species which are in the verge of extinction. The enucleated oocytes of an animal were used to produce a cloned embryo. In the case of extinct breeds cryopreserved DNA, naturally occurring samples or preserved tissues could possibly be used providing an appropriate surrogate dam can be found. The success rate of cloning is very less due to the decreased efficiency. 90% of nuclear transfers turn out to be unproductive. Added to this, cloned animals tend to have more compromised immune function and higher rates of infection, tumor growth and other disorders. There are reports that cloned mice live in poor health and die early. Studies have shown that about 4% of genes function abnormally. The abnormalities do not arise from mutations in the genes but from changes in the normal activation or expression of certain genes (Van Eenennaam *et al.*, 2007).

Also, it is not clear whether the nuclear reprogramming that takes place during the cloning process has any influence over the animal food products that arise from cloned farm animals.

Problems frequently encountered with cloning: Placental abnormalities, fetal overgrowth, prolonged gestation, stillbirth, hypoxia, respiratory failure and circulatory problems, lack of post-natal vigour, increased body temperature at birth, malformations in the urogenital tract (hydronephrosis, testicular hypoplasia), malformations in liver and brain, immune dysfunction, lymphoid hypoplasia, anaemia, thymic atrophy and bacterial and viral infection.

NUCLEAR TECHNIQUES IN ANIMAL REPRODUCTION

In recent years, livestock productivity has been increased by improved reproduction. Various techniques have been developed and refined to obtain a large number of offspring from genetically superior animals or obtain offspring from infertile animals. These techniques include: artificial insemination, cryopreservation of gametes or embryos, induction of multiple ovulations, embryo transfer, *in vitro* fertilization, sex determination of sperm or embryos, nuclear transfer, cloning, etc.

Nuclear transfer: Nuclear Transfer (NT), has numerous potential applications and considerable impact, mainly in agriculture, medicine, pharmacy and fundamental biology. In addition, somatic cell nuclear transfer is the most efficient alternative to produce large transgenic animals (Iguma *et al*., 2005). The potential applications of Somatic Cell Nuclear Transfer (SCNT) technique have received much attention since the first births of cloned animals were reported in various domestic species, including sheep (Wilmut *et al*., 1997), cattle (Kato *et al*., 1998), goat (Baguisi *et al*., 1999), pig (Onishi *et al*., 2000; Polejaeva *et al*., 2000) and more recently, horse (Galli *et al*., 2003). With the nuclear transfer technology one can now create some domestic animals with specific genetic modifications. An ever-expanding variety of cell types have been successfully used as donors to create the clones. Both cell fusion and microinjection are successfully being used to create these animals. However, it is still not clear which stage(s) of the cell cycle for donor and recipient cells yield the greatest degree of development (Kuhholzer and Prather, 2000).

In agriculture, there is current interest in cloning selected livestock that are rare or that have desired genetic traits, such as disease resistance, improved meat quality or yield, or increased milk production. These cloned livestock would be used in breeding programs to incorporate their positive genetic traits into herds more quickly than is possible through conventional breeding and artificial insemination. Developmental biologists in agrobiotechnology have been trying for years to find conditions under which nuclear material from somatic diploid cells could be used as pluripotent source of genetic information. If the donor cell could be genetically manipulated in cell culture using standard stable DNA transfection protocols, then transgenic farm animals would be a common as transgenic mice (Dinnyes and Szmolenszky, 2005). Since, the first mammals to be cloned from cultured differentiated cells (Campbell *et al*., 1996) and the birth of Dolly, the first mammal derived from an adult somatic cell (Wilmut *et al*., 1997) progress was fast.Nuclear replacement efficiency varies among species (Polejaeva *et al*., 2000; Onishi *et al*., 2000).

Somatic cell nuclear transfer is characterized by a series of developmental abnormalities, the so called cloning-syndrome (Dinnyes and Szmolenszky, 2005). The incidence of these anomalies varies according to the species, genotype, donor cell status, or specific aspects of the nuclear transfer and culture protocols used. Despite the present inefficiencies, mouse nuclear replacement experiments have proven that this method is already capable of improving the efficiency of transgenic production compared to traditional methods (Wakayama *et al*., 2001; Tamashiro *et al*., 2002). Nuclear transfer is a fast developing technique, far from being optimized. Evolution in the efficiency and applicability in new species is expected. However, it is not clear how much of an improvement can be achieved by simply optimizing the present protocols. Dinnyes and Szmolenszky (2005) opined that revolutionary changes in our understanding of the reprogramming of the nuclear material, organization of chromatin and epigenetic changes are necessary to allow proper control of the procedures and to avoid epigenetic aberrations in the progeny.

The potential benefits of nuclear transfer are: (1) All animals born will be transgenic, (2) Faster development of a production flock or herd, significantly reducing the time to production of protein or peptide. Where it would normally require 44 months to reach production flock status in sheep, (78 months in cows), nuclear transfer technology can reach production flock status in 18 months for sheep, (33 months for cows); (3) Earlier availability of product, accelerating progress to clinical trials; (4) The selective removal of gene(s), replacing them with the desired human equivalents; (5) Increased expression levels of product (by targeting inserted genes

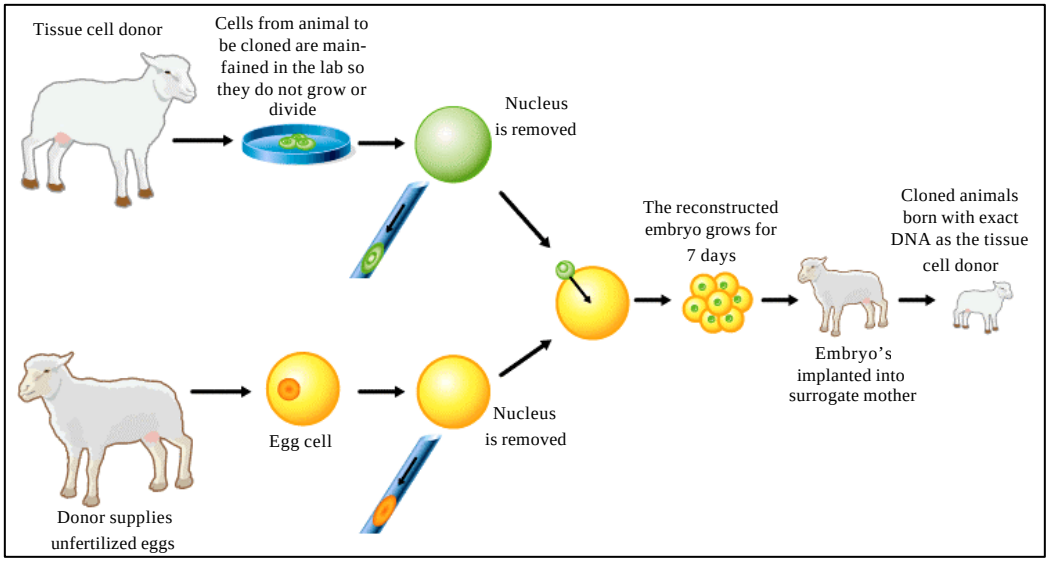

Fig. 6: Pictorial representation of nuclear transfer

to sites of high expression); (6) Cellular-level analysis of clones allowing pre-selection for optimal protein expression. Somatic cell cloning by nuclear transfer is a relatively new technology with many potential applications. However, at the current stage of development, the reprogramming of epigenetic inheritance by nuclear transfer is still incomplete. Further efforts and new paradigms are needed to perfect this technology and extend it to its fullest potential. Various strategies have been used to improve the efficiency of nuclear transfer, however, significant breakthroughs are yet to happen (Tian *et al*., 2003). Figure 6 describes the pictorial representation of nuclear transfer.

Gene transfer: Gene transfer is to transfer a gene from one DNA molecule to another DNA molecule. There are two basic approaches presently in use for inserting DNA into vertebrate germ line cells-transfection and infection with retrovirus vectors. A third approach based on the use of mobile genetic elements, has been commonly used for insects and is being explored for germ-line modification of vertebrates (Ma and Chen, 2005). Some of the more promising alternative strategies such as sperm-mediated gene transfer, restriction enzyme-mediated integration, metaphase II transgenesis and a new twist on retrovirus-mediated gene transfer have also been recently identified (Wall, 2002). To be functional, the integrated gene must be expressed and regulated appropriately. Thus, the gene to be transferred must be accompanied by the appropriate promoter and regulatory sequences. Some genes require an enhancer that may be located far from the promoter. Gene that is introduced into the cell from an external source is called transgene. A fertile animal that carries an introduced gene(s) in its germ line is called transgenic animal. The introduction of gene(s) into animal cells that leads to the transmission of the input transgene to successive generation is called transgenesis.

Marker-assisted selection: The potential benefits of using markers linked to genes of interest in breeding programmes, thus moving from phenotype based towards genotype-based selection, have been obvious for many decades. However, realization of this potential has been limited by the lack of markers. With the advent of DNA-based genetic markers in the late 1970s, the situation changed and researchers could, for the first time, begin to identify large numbers of markers dispersed throughout the genetic material of any species of interest and use the markers to detect associations with traits of interest, thus allowing MAS finally to become a reality (Andersson, 2001).

Successful application of MAS in breeding programmes requires advances in the following five areas: gene mapping, marker genotyping, QTL (quantitative trait Loci) detection, genetic evaluation and MAS development (Dekkers and Chakraborty, 2001; Maddox, 2005). The first reported map in livestock was for chicken in 1992, which was quickly followed by the publication of maps for cattle, pigs and sheep (Dekkers and Hospital, 2002). Since then, the search for useful markers has continued and further species have been targeted,

including goat, horse, rabbit and turkey. Dekkers (2004) recently reviewed commercial applications of MAS in livestock and noted that several gene or marker tests are available on a commercial basis in different species and for different traits. Specific markers have been identified for selection of different traits such as meat, milk, wool and diseases specific to livestock (Olivier *et al*., 2005).

Molecular marker maps, the necessary framework for any MAS programme, have been constructed for the majority of agriculturally important species, although the density of the maps varies considerably among species (Marshall *et al*., 2004; Maddox, 2005). Despite the considerable resources that have been invested in this field and despite the enormous potential it still represents, with few exceptions, MAS has not yet delivered its expected benefits in commercial breeding programmes for livestock in the developed world. When evaluating the potential merits of applying MAS as a tool for genetic improvement in developing countries, some of the issues that should be considered are its economic costs and benefits, its potential benefits compared with conventional breeding or with application of other biotechnologies and the potential impact of Intellectual Property Rights (IPRs) on the development and application of MAS.

Sex determination of sperm: There has been great interest in sexing sperm ever since AI was practiced widely (Seidel and Garner, 2002; Seidel, 2003). The basic principles are simple; the X-sperm contains more DNA than the Y-sperm. Although this difference is small, by attention to details, it is possible to measure DNA content of individual sperm with sufficient accuracy to distinguish between X- and Y-sperm (Garner and Siedel, 2003). A number of approaches to the sexing of semen have been attempted and several have been reported as successful. However, the only method of semen sexing that has shown any promise has been the sorting of spermatozoa according to the DNA content, by means of flow cytometry. Flow cytometric technology is widely used for sexing sperm in mammalian species. Differences in DNA content have provided both a method to differentiate between these sex-determining gametes and a method to sort them that can be used for predetermining sex in mammals (Garner, 2006). The precision of this DNA measurement is such that the difference in DNA content between mammalian X-and Y-chromosome bearing sperm of a variety of mammals has been determined. Successful sperm sexing of various species must take into account the relative susceptibilities of gametes to the stresses that occur during sexing. Sperm sexing by high speed flow cytometry has been one of the most significant new technologies for artificial breeding of livestock developed in the twentieth century (Seidel, 2007). A very recent and most exciting development in this field is the establishment of reverse sex-sorting technology for the utilization of frozen ram and bull semen (Hollinshead *et al*., 2003; Underwood *et al*., 2009). These results demonstrate that frozen-thawed ram and bull sperm can be sex-sorted for either immediate or future use in an IVF system after re-cryopreservation.

Sperm Sexing Technology (SST) will enable the producers of livestock to predetermine the sex of offspring prior to conception, thereby maximizing productivity, profitability and genetic potential. In virtually every sector of commercial animal breeding there is the clear preference for one sex over the other. The technology does not involve genetic modification and is non-invasive. The ability to sex semen has a large potential for commercialization; thus, much of the research to develop and refine sperm sexing technology has been conducted in the private sector. However, along with the new sexing technology, it is very important to meet the need to develop new semen storage and processing methods, so that the sexed sperm can be utilized for AI or for *in vivo* and *in vitro* embryo production (Seidel, 2007; Beilby *et al*., 2009). The potential application of sexed sperm technology is (1) to obtain more female calves; (2) to obtain male calves from the very best cows in the herd to use as breeding bulls; (3) One dose of sexed sperm can be used to produce many embryos through *in vitro* fertilization.

Sex determination of embryos: Producers of domestic livestock strive to improve genetic influences in their herds. This requires identification and propagation of animals that demonstrate desirable characteristics. The more animals available from which to select, the greater the opportunity to discover high-performance animals. Predetermination of the sex of offspring would provide a greater number of males or females from which to select the top individuals that will contribute the genetics to the next generation (Plummer and Beckett, 2006). The embryo transfer technology represents a powerful tool for the acceleration of various breeding programs in cattle. Known sex of embryos produced for use in ET programs can more effectively help to manage producer resources because more heifer calves per ET can be produced. This approach can improve the genetic potential of cattle herds in shorter time intervals (Lopatarova *et al*., 2008). Sexing of embryos before transfer and implanting has great potential for the livestock industry. Manipulating the sex of offspring has been a dream of livestock industry for decades (Chen *et al*., 1999).

Embryo sexing has been attempted by a variety of methods, including cytogenetic analysis, assays for X-linked enzyme activity, analysis of differential development rates, detection of male-specific antigens and the use of Y-specific DNA probes. In DNA amplification techniques, the probe itself is not used. The sequence information generated from the identification and cloning of the probe is used instead. Several methods have now been reported for mammalian sex determination as following (1) Using polymerase chain reaction (PCR) to amplify a Y-chromosome; (2) PCR-based genotyping; (3) PCR-mediated approach uses three sets of primers for sex determination of pre-implantation bovine embryos; (4) rapid sexing method for preimplantation embryos of bovine using Loop-Mediated Isothermal Amplification (LAMP) reaction (Zoheir and Allam, 2010).

Since, the development of DNA amplification techniques, particularly the Polymerase Chain Reaction (PCR), these techniques have been applied to numerous situations in which the analysis of rare sequences is desired (Peura *et al*., 1991). It was the identification of bovine Y-chromosome specific DNA probes and the subsequent development of DNA amplification techniques by PCR, that made the possibility of sexing embryos into a reality. Using DNA analysis to analyse the sex of embryos has been shown to be reliable. The removal of a few cells in this procedure, caused very little trauma to the embryos. It did not alter developmental potential *in vitro*. The procedure has been shown to be sensitive, accurate and efficient and pregnancy rates have not been affected, compared to those after transfer of fresh embryos without sexing. Several commercial Polymerase Chain Reaction (PCR) kits are available which uses the primers specific to the Y-Chromosome Determinant (YCD) to determine sex of the embryos. The PCR product was detected by UV light in agarose gel with ethidium bromide and the embryos were scored as Y-chromosome determinant positive (male) or Y-chromosome determinant negative (Herr *et al*., 1995; Lopatarova *et al*., 2008).

Radioimmunoassay impact on animal reproduction: Early pregnancy detection and prediction of the number of fetus would be profitable for livestock breeders because it enables them to adjust nourishment of pregnant animals according to the individual needs in order to prevent health problems around parturition. RIA technique for early non-pregnancy diagnosis can be integrated in to AI programmes in order to increase their effectiveness, reduce the unproductive period of dairy cows and increase the economic benefits to farmers. Progesterone RIA presented a very reliable tool for early diagnosis of non-pregnancy and infertility. The use of progesterone measurement for early diagnosis of non-pregnant cows has a high level of accuracy and can improve the efficiency of AI delivery, reduce calving intervals and improve milk and meat production from the dairy industry. Further monitoring reproduction through RIA of progesterone was cost-effective in improving reproductive efficiency. In addition RIA technique can be used to detect physiologically relevant increases in serum FSH related to emergence of each new wave of follicle growth (Crowe *et al*., 1997). Highly sensitive RIA using I-125 has been developed and used to measure the minute quantities of reproductive and other hormones circulating in the blood that control reproduction. Development of RIA technology has paved way for estimation of almost all reproductive hormones which in turn depicts the reproductive status of the animals. RIA has made it possible to determine when animals are ready for breeding, diagnose pregnancy earlier than would be otherwise possible, check whether animals have been inseminated at the correct time, devise corrective measures for reproductive disorders and improve the efficiency of artificial insemination and embryo transfer programs (Dargie, 1990).

CONCLUSIONS

There is general agreement among animal scientists that the single most important cause of economic losses in the animal industries is reproductive inefficiency. The new advanced technologies described in this review have the potential to add to the desired developments in farm animal breeding. Further developments in advanced reproductive technologies is the hour of need as the farm animals offers greater future potential for economic development. These technologies will be valuable research tools enabling scientists to gain deeper understanding of livestock reproduction. The greater challenge lies ahead for animal researchers is to integrate and potentially exploit these novel technologies in a society-friendly manner. Accepting this challenge and working towards achieving such targets should enable us to reap maximum benefits from the farm animal sector.

REFERENCES

Adam, C.L., Z.A. Archer and D.W. Miller, 2003. Leptin actions on the reproductive neuroendocrine axis in sheep. Reprod. Suppl., 61: 283-297.

Agarwal, S.K., K. Vogel, S.R. Weitsman and D.A. Magoffin, 1999. Leptin antagonizes the insulin-like growth factor-I augmentation of steroidogenesis in granulosa and theca cells of the human ovary. J. Clin. Endocrinol. Metab., 84: 1072-1076.

Andersson, L., 2001. Genetic dissection of phenotypic diversity in farm animals. Nat. Rev. Genet., 2: 130-138.

Arreguin-Arevalo, J.A., C.A. Lents, T.A. Farmerie, T.M. Nett and C.M. Clay, 2007. KiSS-1 peptide induces release of LH by a direct effect on the hypothalamus of ovariectomized ewes. Anim. Reprod. Sci., 101: 265-275.

Baguisi, A., E. Behboodi, D.T. Melican, J.S. Pollock and M.M. Destrempes *et al.*, 1999. Production of goats by somatic cell nuclear transfer. Nat. Biotechnol., 17: 456-461.

Baratta, M. and C. Tamanini, 1992. Effect of melatonin on the *in vitro* secretion of progesterone and estradiol 17b by ovine granulose cells. Acta Endocrinol., 127: 366-370.

Barb, C.R. and R.R. Kraeling, 2004. Role of leptin in the regulation of gonadotropin secretion in farm animals. Anim. Reprod. Sci., 82: 155-167.

Barb, C.R., G.J. Hausman and T.G. Ramsay, 2006. Leptin in Farm Animals. In: Leptin. Endocrinology Update Series, Castracane, V.D. and M.C. Henson (Eds.). Springer Publishing Group, New York, NY, pp: 263-308.

Beilby, K.H., C.G. Grupen, P.C. Thomson, W.M.C. Maxwell and G. Evans, 2009. The effect of insemination time and sperm dose on pregnancy rate using sex-sorted ram sperm. Theriogenology, 71: 829-835.

Betteridge, K.J., 2003. A history of farm animal embryo transfer and some associated techniques. Anim. Reprod. Sci., 79: 203-244.

Blackburn, H.D., 2004. Development of national animal genetic resource programs. Reprod. Fertil. Dev., 16: 27-32.

Blasco, A., 2008. The role of genetic engineering in livestock production. Livest. Sci., 113: 191-201.

Brancia, C., P. Nicolussi, P. Cappai, G.L. Corte, R. Possenti and G. Ferri, 2005. Differential expression and seasonal modulation of VGF peptides in sheep pituitary. J. Endocrinol., 186: 97-107.

Brannian, L.J.D., Y. Zhao and M. McElroy, 1999. Leptin inhibits gonadotrophin-stimulated granulosa cell progesterone production by antagonizing insulin action. Hum. Reprod., 14: 1445-1448.

Byatt, J.C., W.C. Warren, P.J. Eppard, N.R. Staten, G.G. Krivi and R.J. Collier, 1992.. Ruminant placental lactogens: Structure and biology. J. Anim. Sci., 70: 2911-2923.

Campbell, K.H.S., J. McWhir, W.A. Ritchie and I. Wilmut, 1996. Sheep cloned by nuclear transfer from a cultured cell line. Nature, 380: 64-66.

Campbell, B.K., C.G. Guitierrez, D.G. Armstrong, R. Webb and D.T. Baird, 2000. Leptin: *In vitro* and *in vivo* evidence for direct effects on the ovary in monovulatory ruminants. Human Reprod., 14: 15-16.

Chaucer, G., 2003. What of animal cloning? Can. Vet. J., 44: 1343-1400.

Chen, C.M., C.L. Hu, C.H. Wang, C.M. Hung and H.K. Wu *et al.*, 1999. Gender determination in single bovine blastomeres by polymerase chain reaction amplification of sex-specific polymorphic fragments in the amelogenin gene. Mol. Reprod. Dev., 54: 209-214.

Chieng, B. and J.T. Williams, 1998. Increased opioid inhibition of GABA release in nucleus accumbens during morphine withdrawal. J. Neurosci., 18: 7033-7039.

Cosgrove, R., F. de Rensis and G.R. Foxcroft, 1993. Opioidergic pathways in animal reproduction: Their role and effects of their pharmacological control. Anim. Reprod. Sci., 33: 373-392.

Crowe, M.A., V. Padmanabhan, N. Hynes, S.J. Sunderland, W.J. Enright, I.Z. Beitins and J.F. Roche, 1997. Validation of a sensitive radioimmunoassay to measure serum follicle-stimulating hormone in cattle: correlation with biological activity. Anim. Reprod. Sci., 48: 123-136.

Cunningham, M.J., D.K. Clifton and R.A. Steiner, 1999. Leptins actions on the reproductive axis: Perspectives and mechanisms. Biol. Reprod., 60: 216-222.

Dargie, J.D., 1990. Helping Small Farmers to Improve their Livestock. International Atomic Energy Agency, Vienna.

Dekkers, J.C.M. and F. Hospital, 2002. The use of molecular genetics in the improvement of agricultural populations. Nat. Revs. Genet., 3: 22-32.

Dekkers, J.C.M. and R. Chakraborty, 2001. Potential gain from optimizing multi-generation selection on an identified quantitative trait locus. J. Anim. Sci., 79: 2975-2990.

Dekkers, J.C.M., 2004. Commercial application of marker-and gene-assisted selection in livestock: Strategies and lessons. J. Anim. Sci., 82: E313-E328.

Dinnyes, A. and A. Szmolenszky, 2005. Animal cloning by nuclear transfer: State-of-the-art and future perspectives. Acta Biocimica Polonica, 52: 585-588.

Dupont, J., V. Maillard, S. Coyral-Castel, C. Ramé and P. Froment, 2010. Ghrelin in female and male reproduction. Int. J. Peptides, 10.1155/2010/158102

Dyer, R.G., N. Parvizi, S. Hollingsworth, S. Mansfield, R.P. Heavens, R.J. Bicknell and D.J.S. Sirinathsinghji, 1991. Opioid binding in the rostral hypothalamus is reduced following lesion of the ventral noradrenergic tract in female rats. Exp. Brain Res., 85: 230-235.

Funes, S., J.A. Hedrick, G. Vassileva, L. Markowitz and E.L. Gustafson, 2003. The KiSS-1 receptor GPR54 is essential for the development of the murine reproductive system. Biochem. Biophys. Res. Commun., 312: 1357-1363.

Gallegos-Sanchez, J., B. Malpaux and J.C. Thiery, 1998. Control of pulsatile LH secretion during seasonal anoestrus in the ewe. Reprod. Nutr. Dev., 38: 3-15.

Galli, C., I. Lagutina, G. Crotti, S. Colleoni and G. Lazzari *et al*., 2003. Pregnancy: A cloned horse born to its dam twin. Nature, 424: 635-635.

Gangula, P.R.R., C. Thota, S.J. Wimalawansa, R.D. Bukoski and C. Yallampalli, 2003. Mechanisms involved in calcitonin gene-related peptide-induced relaxation in pregnant rat uterine artery. Biol. Reprod., 69: 1635-1641.

Garner, D.L. and G.E. Seidel Jr, 2003. Past, present and future perspectives on sexing sperm. Can. J. Anim. Sci., 83: 375-384.

Garner, D.L., 2006. Flow cytometric sexing of mammalian sperm. Theriogenology, 65: 943-957.

Greives, T.J., L.J. Kriegsfeld, J.E. Bentley, K. Tsutsui and G.E. Demas, 2008. Recent advances in reproductive neuroendocrinology: A role for RFamide peptides in seasonal reproduction? Proc. R. Soc. B., 275: 1943-1951.

Herr, C.M., T. Steel, A. Lascelles and K.C. Reed, 1995. Instruction Manual: Splitting, Biopsy and Rapid Sexing of Cattle Embryos. AB Technology, Inc., Pullmann, WA., pp: 58.

Hollinshead, F.K., L. Gillan, J.K. O'Brien, G. Evans and W.M.C. Maxwell, 2003. *In vitro* and *in vivo* assessment of functional capacity of flow cytometrically sorted ram spermatozoa after freezing and thawing. Reprod. Fertil. Dev., 15: 351-359.

Iguma, L.T., F.C.L. Sharon, E.O. Melo, M.M. Franco and I. Pivato *et al*., 2005. Development of bovine embryos reconstructed by nuclear transfer of transfected and non-transfected adult fibroblast cells. Genet. Mol. Res., 4: 55-66.

Jadav, P.V., D.M. Patel, F.S. Kavani and A.J. Dhami, 2010. Gnrh and its applications in bovine reproduction. J. Adv. Dev. Res., 1: 74-80.

Kato, Y., T. Tetsuya, Y. Sotomaru, K. Kurokawa and J. Kato *ert al*., 1998. Eight calves cloned from somatic cells of a single adult. Science, 282: 2095-2098.

Khanal, D.R. and R.C. Munankarmy, 2009. Nuclear and related techniques for enhancing livestock and agriculture productivity. J. Agric. Environ., 10: 150-155.

Kuhholzer, B. and R.S. Prather, 2000. Advances in livestock nuclear transfer. Proc. Soc. Exp. Biol. Med., 224: 240-245.

Lopatarova, M., S. Cech, P. Krontorad, L. Holy, J. Hlavicova and R. Dolezel, 2008. Sex determination in bisected bovine embryos and conception rate after the transfer of female demi-embryos. Vet. Med., 53: 295-603.

Ma, H. and G. Chen, 2005. Gene transfer technique. Nat. Sci., 3: 25-31.

Ma, W.M., Y.Q. Qian, M.R. Wang, F. Yang and W.J. Yang, 2010. A novel terminal ampullae peptide is involved in the proteolytic activity of sperm in the prawn, *Macrobrachium rosenbergii*. Reproduction, 140: 235-245.

Maddox, J.F., 2005. A presentation of the differences between the sheep and goat maps. Genet. Sel. Evol., 37: S1-S10.

Mapletoft, R.J. and J.F. Hasler, 2005. Assisted reproductive technologies in cattle: A review. Rev. Sci. Tech. Off. Int. Epiz., 24: 393-403.

Marshall, K.J., J.H.J. van der Werf and J. Henshall, 2004. Exploring major gene-marker phase typing strategies in marker assisted selection schemes. Anim. Sci., 78: 213-227.

Mather, J.P., T.K. Woodruff and L.A. Krummen, 1992. Paracrine regulation of reproductive function by inhibin and activin. Proc. Soc. Exp. Biol. Med., 201: 1-15.

Meenambigai, T.V. and V. Sejian, 2011. Insights into embryonic stem cells of bovines. Asian J. Anim. Sci. 5: 1-18.

Meenambigai, T.V., V.V. Vardhini, A. Palanisamy, S. Sathesh Kumar, K. Brindha and K. Kumanan, 2009. Expression of gap junctional connexin 43 gene in cumulus oocyte complexes in Buffalo cumulus oocyte complexes. Ind. Vet. J., 86: 890-892.

Miller, D.W., J.L. Harrison, E.J. Bennett, P.A. Findlay and C.L. Adam, 2007. Nutritional influences on reproductive neuroendocrine output: Insulin, leptin and orexigenic neuropeptide signaling in the ovine hypothalamus. Endocrinol., 148: 5313-5322.

Montaldo, H.H., 2006. Genetic engineering applications in animal breeding. Electron. J. Biotechnol., 9: 157-170.

Naqvi, S.M.K., A. Joshi, G.K. Das and J.P. Mittal, 2001. Development and application of ovine reproductive technologies: An Indian experience. Small Rumin. Res., 39: 199-208.

Naqvi, S.M.K., R. Gulyani, A. Joshi, G.K. Das and J.P. Mittal, 2002. Effect of dietary regimens on ovarian response and embryo production of sheep in tropics. Small Rumin. Res., 46: 167-171.

Ohleth, K.M., Q. Zhang, J.A. Lenhart, P.L. Ryan and C.A. Bagnell, 1999. Trophic effects of relaxin on reproductive tissue: Role of the IGF system. Steroids, 64: 634-639.

Olivier, J.J., S.W.P. Cloete, S.J. Schoeman and C.J.C. Muller, 2005. Performance testing and recording in meat and dairy goats. Small Rumin. Res., 60: 83-93.

Onishi A, M. Iwamoto, T. Akita, S. Mikawa and K. Takeda *et al*., 2000. Pig cloning by microinjection of fetal fibroblast nuclei. Science, 289: 1188-1190.

Osugi, T., K. Ukena, G.E. Bentley, S.O'Brien, I.T. Moore, J.C. Wingfield and K. Tsutsui, 2004. Gonadotropin-inhibitory hormone in Gambels white-crowned sparrow (*Zonotrichia leucophrys gambelii*): cDNA identification, transcript localization and functional effects in laboratory and field experiments. J. Endocrinol., 182: 33-42.

Pang, S.F., L. Li, E.A. Ayre, C.S. Pang and P.P.N. Lee *et al*., 1998. Neuroendocrinology of melatonin in reproduction: Recent developments. J. Chem. Neuroanat., 14: 157-166.

Parvizi, N., 2000. Neuroendocrine regulation of gonadotropins in the male and the female. Anim. Reprod. Sci., 60-61: 31-47.

Paterson, L., P. DeSousa, W. Ritchie, T. King and I. Wilmut, 2003. Application of reproductive biotechnology in animals: Implications and potentials applications of reproductive cloning. Anim. Reprod. Sci., 79: 137-143.

Payan-Carreira, R., P. Rodrigues and P.R.F. Carvalho, 2006. Chromogranin-A expression in the bovine testis. Anim. Reprod. Sci., 96: 146-153.

Peura, T., J.M. Hyttinen, M. Turunen and J. Janne, 1991. A reliable sex determination assay for bovine preimplantation embryos using the polymerase chain reaction. Theriogenology, 35: 547-555.

Plummer, W.E. and D. Beckett 2006. Development of successful sex determination method of bovine embryos utilizing embryo biopsy and pcr. California State University Agricultural Research Initiative Final Report.

Polejaeva, I.A. and K.H. Campbell, 2000. New advances in somatic cell nuclear transfer: Application in transgenesis. Theriogenology. 53: 117-126.

Polejaeva, I.A., S.H. Chen, T.D. Vaught, R.L. Page and J. Mullins *et al*., 2000. Cloned pigs produced by nuclear transfer from adult somatic cells. Nature, 407: 86-90.

Popa, S.M., D.K. Clifton and R.A. Steiner, 2008. The role of Kisspeptins and GPR54 in the neuroendocrine regulation of reproduction. Annu. Rev. Physiol., 17: 213-238.

Quaynor, S., L. Hu, P.K. Leung, H. Feng, N. Mores, L.Z. Krsmanovic and K.J. Catt, 2007. Expression of a functional G protein-coupled receptor 54-Kisspeptin autoregulatory system in hypothalamic gonadotropin-releasing hormone neurons. Mol. Endocrinol., 21: 3062-3070.

Roche, J.F., 1996. Control and regulation of folliculogenesis-a symposium in perspective. Rev. Reprod., 1: 19-27.

Sandoe, G.T.P., 2005. The science and technology of farm animal cloning. Summary of Project Report 6. http://www.sl.kvl.dk/cloninginpublic/index-filer/THESCIENCEANDTECHNOLOGYSummary.pdf

Seidel Jr, G.E., 2003. Sexing mammalian sperm-intertwining of commerce, technology and biology. Anim. Reprod. Sci., 79: 145-156.

Seidel, Jr. G.E. and D.L. Garner, 2002. Current status of sexing mammalian spermatozoa. Reproduction, 124: 733-743.

Seidel, Jr., G.E., 2007. Overview of sexing sperm. Theriogenology, 68: 443-446.

Seminara, S.B., S. Messager, E.E. Chatzidaki, R.R. Thresher and J.S. Acierno Jr *et al*., 2003. The GPR54 gene as a regulator of puberty. New England J. Med., 349: 1614-1627.

Smith, J.T. and I.J. Clarke, 2007. Kisspeptin expression in the brain: Catalyst for the initiation of puberty. Rev. Endocr. Metab. Disord., 8: 1-9.

Srivastava, R.S. and V. Sejian, 2010. Recent Advances in Neuronal Stem Cell Research. In: Short Course on 'Recent Advances in Stem Cell Research, Bag, S., A.C. Majumdar, B.C. Das and G. Tarusharma (Eds.). Centre of Advanced Faculty Training in Veterinary Physiology, Division of Physiology and Climatology, Indian Veterinary Research Institute, Izatnagar, Bareilly, India, pp: 57-60.

Tamashiro, K.L., T. Wakayama, H. Akutsu, Y. Yamazaki and J.L. Lachey *et al*., 2002. Cloned mice have an obese phenotype not transmitted to their offspring. Nat. Med., 8: 262-267.

Taya, K., H. Kaneko, T. Takedomi, H. Kishi and G. Watanabe, 1996. Role of inhibin in the regulation of FSH secretion and folliculogenesis in cows. Anim. Reprod. Sci., 42: 563-570.

Tian, X.C., C. Kubota, B. Enright and X. Yang, 2003. Cloning animals by somatic cell nuclear transfer-biological factors. Reprod. Biol. Endocrinol., 1: 98-98.

Underwood, S.L., R. Bathgate, W.M.C. Maxwell and G. Evans, 2009. *In vitro* characteristics of frozen-thawed sex-sorted bull spermatozoa after re-freezing or incubation at 15 or 37°C. Theriogenology, 72: 1001-1008.

Vajta, G. and M. Gjerris, 2006. Science and technology of farm animal cloning: State of the art. Anim. Reprod. Sci., 92: 211-230.

Van Eenennaam, A.L., R.L. Weaber, D.J. Drake, M.C.T. Penedo, R.L. Quaas, D.J. Garrick and E.J. Pollak, 2007. DNA-based paternity analysis and genetic evaluation in a large, commercial cattle ranch setting. J. Anim. Sci., 85: 3159-3169.

Velazquez, M.A., L.J. Spicer and D.C. Wathes, 2008. The role of endocrine insulin-like growth factor-I (IGF-I) in female bovine reproduction. Domestic Anim. Endocrinol., 35: 325-342.

Vigo, E., J. Roa, R. Pineda, J.M. Castellano and V.M. Navarro *et al*., 2007. Novel role of the anorexigenic peptide neuromedin U in the control of LH secretion and its regulation by gonadal hormones and photoperiod. Am. J. Physiol. Endocrinol. Metab., 293: E1265-E1273.

Wakayama, T., V. Tabar, I. Rodriguez, A.C. Perry, L. Studer and P. Mombaerts, 2001. Differentiation of embryonic stem cell lines generated from adult somatic cells by nuclear transfer. Science, 292: 740-743.

Wall, R.J., 2002. New gene transfer methods. Thereogenology, 57: 189-201.

Walther, T. and H. Stepan, 2004. C-type natriuretic peptide in reproduction, pregnancy and fetal development. J. Endocrinol., 180: 17-22.

Wilmut, I., A.E. Schnieke, J. McWhir, K.L. Kind and K.H.S. Campbell, 1997. Viable offspring derived from fetal and adult mammalian cells. Nature, 385: 810-813.

Wolf, E., W. Schernthaner, V. Zakhartchenko, K. Prelle, M. Stojkovic and G. Brem, 2000. Transgenic technology in farm animals-Progress and perspectives. Exp. Physiol., 85: 615-625.

Wrathall, A.E., H.A. Simmons, D.J. Bowles and S. Jones, 2004. Biosecurity strategies for conserving valuable livestock genetic resources. Reprod. Fertil. Dev., 16: 103-112.

Zachow, R.J., S.R. Weitsman and D.A. Magoffin, 1999. Leptin impairs the synergistic stimulation by transforming growth factor-beta of follicle-stimulating hormonedependent aromatase activity and messenger ribonucleic acid expression in rat ovarian granulosa cells. Biol. Reprod., 61: 1104-1109.

Ziecik, A.J., W. Sienkiewicz, S. Okrasa, H. Kalamarz, M. Lakomy and R.R. Kraeling, 1999. Concentration and distribution of neuropeptide y, galanin, β-endorphin, vasoactive intestinal peptide and gonadotrophin-releasing hormone in the hypothalamus of gilts during oestrogen-induced surge secretion of luteinizing hormone. Reprod. Domest. Anim., 34: 477-487.

Zoheir, K.M.A. and A.A. Allam, 2010. A rapid method for sexing the bovine embryo. Anim. Reproduc. Sci., 119: 92-96.