Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(4): 62-66

Received: 14-05-2018
Accepted: 19-06-2018

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Recent advances in reproductive biotechnologies in small ruminants

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Abstract
The biotechniques in reproduction have made significant contribution in increasing the reproductive efficiency, global transport and multiplication of genetic material, and conservation of unique genetic material for use in future. Animal biotechnology encompasses the application of science and engineering principles to the processing or production of materials to provide services or goods for human use. These techniques are artificial induction of breeding characteristics in female, artificial insemination (AI) using either fresh, frozen or sexed semen, Embryo transfer using fresh or frozen *in vivo* or *in vitro* produced embryos, cloning using somatic cell nuclear transfer (SCNT) procedures and production of transgenic animals. Artificial induction of estrus involves the use of recombinant exogenous progesterone alone or together with gonadotropins. Through artificial insemination selected male can pass his merit to thousands of females. By embryo production and transfer technology we can produce more number of offspring from a superior female, in a minimum time and cloning involves somatic cell nuclear transfer procedures and production of transgenic animals.

Keywords: Artificial insemination, embryo transfer technique, sex semen, cloning

Introduction
India has a more than 130 million human population [1] and agriculture is considered as the backbone of food security of a majority of Indians. It is speculated that Indian population is likely to reach 150 million by 2030 [2]. In the present scenario, India produces about 230 million tones of cereals that meet the food security of only 88.46 percent of Indian [3]. If we calculate the future food requirements, the need of farm animals are often overlooked. Although, milk is most likely consumed, now Indian population has increased the consumption of meat. Thus, livestock plays a significant role in food security.

In India, domestic goat Capra hircus and sheep Ovis aries is known as “poor man's cow” for its excellent quality of milk, meat, fibre, and skin [4]. Moreover, these small ruminants play an essential role in the food, nutritional as well as the economical security of the rural Indian population [5, 6]. Although current census is not available, India has a 135.2 million of goat and 65.1 million of sheep population [7]. As per the data, during the year 2004-05, per capita consumption of goat or sheep meat in rural and urban India was 0.047 and 0.070 Kg/month, respectively [8]. Surprisingly, current goat and sheep population is not sufficient enough to maintain the demand. The reason behind a poor population of goat and sheep is their poor reproductive performance [9]. Short and seasonal breeding characteristic of goat and sheep’s again contributes to the limited growth of these small ruminants [10]. Furthermore, caprine uterus exhibits a higher prevalence of genital lesions (14.6%), and endometritis and ovarian cysts are the most common infections which cause abortion, small litter size and infertility in ewes [11-13]. Together, the problems associated with buck reproduction, the investigation of female infertility in small ruminants is not that easy as cows. Moreover, the seasonal pattern of breeding of these ruminants, it limits the tools that can be used for the reproductive assessment and cuts the available time for remedial measures [14].

From the past few decades several biotechnological tools are employed to solve these issues. These tools include, 1) artificial induction of breeding characteristics in female; 2) artificial insemination (AI) using either fresh, frozen or sexed semen; 3) Embryo transfer using fresh or frozen *in vivo* or *in vitro* produced embryos, 4) cloning using somatic cell nuclear transfer (SCNT) procedures and production of transgenic animals. However, even these biotechnological tool are well developed, except a few cases of AI, these tools are poorly

adapted. Slowly, the field of reproductive biotechnology is making research area for Indian scientist with a belief of their significant benefits for their application within goat and sheep meat industry. The quick idea of the recent development of these biotechnological tools for the management and production of sheep and goats are as follows-

1. Artificial induction of Estrus

Out-of-season breeding of sheep and goat reduces the seasonal fluctuation of herd's milk, meat and fibre production. Moreover, increasing conception rates and litter sizes per year is very important for the meat industry. In the annual patterns of breeding activity, melatonin stimulates dopamine, serotonin and other amino acids in order to modulate the hypothalamic secretion of gonadotropin-releasing hormone [15]. However, during the non-breeding season, the degree of activity of hypothalamic-pituitary-gonadal axis for gradual ovulation gradually decreases [16]. Thus, the biotechnological approach to induce estrus in sheep and goats involves the use of recombinant exogenous progesterone alone or together with gonadotropins. The commonly based method of estrus induction includes controlled internal drug release (CIDR) or intravaginal polyurethane sponges impregnated with recombinant progesterone (P4 or their synthetic analogues medroxyprogesterone, melengestrol and fluorogestone acetate forms) with equine chorionic gonadotropin (eCG) and prostaglandin F2 alfa (PGF2α) or strong estrogenic pharmacologic active substances [17-21].

2. Artificial Insemination (AI)

The AI technology is widely applied to reproductive biotechnology in the world, for its merit of rapid up gradation of the genetic animal through selective breeding and improvement production trait by eliminating lethal alleles. Moreover, the selected male can pass his merit to thousands of females and makes this a far more efficient biotechnology. This can also contribute to increase the number of offspring per year by using female based embryo transfer (ET) technology. This also helps to only produce progeny from a selected female. More recently, the incorporation of sperm sexing technology together with AI tool opened a new window for the production of selected sexed progenies. The sperm sexing technology is a more reliable and economical method of obtaining predetermined sex progeny in animals as compared to other embryo sexing and pre-implantation genetic diagnosis [22-24]. Moreover, the successful birth of sex-selected kids by artificial insemination with sex-sorted Saanen goat spermatozoa [25] and sheep [26, 27] is already evident.

Semen sexing Technology

a) Fluorescence-activated cell sorting (FACS)

Over the past 40 years, considerable efforts have been made to develop a technology to separate X- and Y- bearing spermatozoa, the success is very limited particular in the cattle [28]. The recent and most adopted technology of sperm sexing is based on exclusive DNA content of spermatozoa. X-bearing spermatozoa carries almost 3.4% higher amount of DNA as compared to Y- bearing. So, X and Y-chromosome bearing florescent labelled sperm cells can be separated into fractions by fluorescence-activated cell sorting (FACS) [29]. However, this procedure is very slow, expensive and leads to sub-lethal damages to cells [30] including sheep spermatozoa [31]. This limits the standard insemination procedure of semen deposition in cervix or uterus of recipient female. It is believed that sex-sorted semen should deposit in the oviduct or deep within the uterus to obtain high success rates. In sheep or goat, transcervical deposition of semen is very difficult. In some special cases laparoscopically is required to deposit semen into the uterus. Thus, as compared to cattle's, the oviductal deposition of sex-sorted frozen semen in sheep is not practical in the farmyard.

b) Immunological method of semen sexing

The basis of the immunological method of semen sexing started when it was speculated that, if X- and Y-sperm differ in their genomic DNA content among different species then it must lead to the protein differences as well. Recently, Chen *et al*. [32] reported that X- and Y-bearing bovine sperm differ in expression of at least 31 genes. Among these, in X-sperm 27 were up-regulated and 4 in Y-sperm. This leads to phenotypic variations in X- and Y-sperm proteins. If these proteins are expressed in sperm surface and if one can isolate/identify such a marker(s) then antibodies against that marker together with immunological methods (such as immunofluorescent labelling, Immunoprecipitation and immunotoxicity approaches) can be employed to separate X- and Y-sperm [33]. This can be much more adventurous than conventional FACS method of fast recovery of sex shorted sperms. However, the accuracy of detecting right sperm and possibly of its separation by using specific antibodies is linked to the accuracy of sperm surface protein marker identification and accessibility of antibodies to the selected protein targets [34].

3 Embryo production and transfer technology

Embryo production (EP) from the mother and transfer (ET) to a surrogate mother opened a new way to produce several progenies from a superior female, in a minimum requirement of time. The conventional biotechnological approach of EP and ETT requires to hormonally superovulate the selected females and at the appropriate time (depending upon species and breed of animals) induced to artificial insemination. Further, after few days, from the donor's uterus, week-old *in vivo* derived embryos use to flush out and microscopically examine for number and quality. Finally, good quality embryos are transferred into the lining of the uterus of surrogate mothers. The super ovulation protocol in goat and sheep requires 14 to 17-day progesterone exposure in the form of the vaginal implant and shots of FSH injections starting from two days before the removal of the progesterone-releasing implant. The FSH injections need to continue for a total of 4-6 shots and animals are allowed to breed naturally or by AI. After 6-7 days of embryo development, morula/ blastocyst stage embryos need to flush and stored or transfer to the surrogate. However, in case of sheep embryo recovery can be only done by surgical methods [35]. For embryo transfer, surrogate mothers are prepared simultaneously to superovulation. In sheep and goat, for estrus synchronization on day-1, progesterone-releasing intravaginal sponges (Veramix®, 60mg medroxyprogesterone acetate, Pharmacia & Upjohn, Orangeville, Canada) need to insert into the vagina, and on the 14th day, the sponges need to remove. Same time recipient is required to inject and 300 IU PMSG (Folligon®, Intervet) intramuscularly then 6-7 days post estrus, surgical embryo transfer is then performed on day-22 [36]. It is estimated that up to the year 2004 researchers have transferred at least *in vivo* derived 68,000 sheep embryos, 1,000 goat embryos, worldwide [37].

However, the recent biotechnological approach of EP and

ETT does not require producing embryos in mother. Recent advances made accessible to *in vitro* produce good quality embryos from oocyte obtained from either superovulated mother or from the ovary of slaughtered animals. The *in vitro* production of embryos includes, oocyte collection, grading and selection of best occyte with more cumulus oophorus, *in vitro* maturation (IVM) of selected oocyte, *in vitro* fertilization (IVF) of IVM oocyte, selection of best quality embryo at morula stage, then finally *in vitro* transfer to recipient goat or sheep. Thus recent ET technology increased the reproductive performance of selected females to avoid loss of rare species, good quality genetics or and production. This also facilitates the development of rare and economically important genetic stocks those are important in livestock breed improvement programme and research. This technology has been extensively used to increase genetically superiority of goats and sheep and optimized for its application commercial practice [38-41].

Apart from the use of ET technology for the above-mentioned application, this technique can be used to produce interspecies individuals by facilitated fertilization of oocyte with interspecies sperm. However, the development of *in vitro* fertilized interspecies hybridized embryos arrest at 4-8 cell stage [42]. In case of goats, when it bred to rams, conceive, and pregnancies will survive till the second trimester. Otherwise, it is not happening in reverse cross when buck sperm was unable to fertilize sheep oocytes *in vivo* [43]. In contrast to this, sheep and goat hybrids are born to goats [42].

4. Somatic cell nuclear transfer (Cloning)

The somatic cell nuclear transfer (SCNT) technique involves isolation of intact diploid nucleus of somatic cells, obtained from the organism to be cloned, and is transfer to the enucleated oocyte [44]. The potential applications SCNT technique has received much attention since the first births of cloned animals were reported in various domestic species, including sheep [45], cattle [46], goat [47], pig [48,49] and more recently, horse [50]. This technique was first used to generate sheep Dolly from a mammary epithelial cell [45]. However, SCNT is associated with significant disadvantages of which include the extremely high rate of pregnancy loss, poor survival of infants, and due to incorrect reprogramming and epigenetic inference of nuclear DNA extraordinary incidence of abnormal development.

The possible reason behind this is, the Telomeres, which is a region of repetitive nucleotide sequences present in each end of the chromosome and protects non-specific endo- or exo-nuclease activity at end of chromosome. This also protects fusion of ends with neighboring chromosomes. In case of SCNT where nucleus donor comes from somatic cells, because of successive cell cycle, the length of telomere become comparatively shorter than that of actual gametes. The stage when the length of telomere reaches to a critical length cells enter to the stage of crisis. This stage is associated with development of abnormal phenomenon or cells. However, this condition is limited in natural gamete fusion. This is because, the embryonic cells contains an enzyme namely telomerase that during replication, adds telomere sequence to chromosomal end preventing shortening of its ends [51]. Although reasons are available declare telomere as a culprit, till date sufficient evidence is unavailable as the data on telomere length in case of Dolly cannot be considered. This statement is appropriate with the evidence of existence of other live sheep offsprings produced by SCNT. Downen TX 63 684 (nicknamed Megan) born on 2001 in Plainwell, Michigan is the first cloned goat of USA and was cloned from a top producing Boer goat. In the Middle East's first cloned goat, Hanna was developed in the surrogacy of Bakhtiari goat and born at the Royan Institute in Isfahan, Iran in 2009. Recently, India has produced the world's first pashmina goat clone, at SKUAST, Kashmir (SKUAST), and named Noori.

Conclusion

Among animal scientists, there is general agreement that reproductive inefficiency in livestock's including small ruminants (sheep and goats) is one of the major causes of the food crisis and economic loss in developing countries. This is because, although new advanced biotechnologies methods have potential to add to the desired developments in farm animal breeding, its adaptability is poor in developing countries. The reason for poor adaptability may include the high cost of application, the requirement of highly technical person, poor extension of technologies, education of farmers, and unresponsive behaviour of state and federal governments. Further, the developments of more advanced and economic reproductive technologies can break these barriers and offers the greater future potential for economic development for maximum benefits to the farm animal sector.

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