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Recent advances in reproductive biotechnologies in sheep and goat

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Abstract
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, it has emerged and started to replace the conventional techniques for sustained livestock productivity. Examples of animal biotechnology include creating transgenic animals (animals with one or more genes introduced by human intervention), using gene knock out technology to make animals with a specific inactivated gene and producing nearly identical animals by somatic cell nuclear transfer (or cloning). Such techniques include crossing diverse strains of animals (known as hybridizing) to produce greater genetic variety. The offspring from these crosses then are bred selectively to produce the greatest number of desirable traits. For example, female horses have been bred with male donkeys to produce mules, and male horses have been bred with female donkeys to produce hin,nies, for use as work animals, for the past 3,000 years. This method continues to be used today.

Keywords: Reproduction, sheep, biotechnology

Introduction

Semen Sexing

This technique issued for producing offspring of the desired sex, either male or female It works on the principle of flow cytometric separation of fluorescent-labelled X-chromosome bearing spermatozoa from the sperms carrying fluorescent-labeled Y-chromosome, capable of analysing over 100,000 events (sperms) per second. Accuracy of predicting the sex is between 85% and 95%. Semen sexing can be used for reducing the incidence of sex-linked diseases, besides conservation of superior and rare animals. Limitations of this technique is variety of damages *viz*., destabilization of sperm membrane and capacitation-like changes thereby reducing lifespan of sorted spermatozoa in the female genital tract.

Table 1.

Place	Species	Success Rate
Faculty of Veterinary Science, The University of Sydney, Sydney, NSW, Australia (Bathgate *et al.*)	Sheep	83% of kids being of the expected sex
Fort Collins, Colorado, USA (Hollinshead *et al.*)	Sheep/Goat	26 of the 30 (86.7%) lambs derived from sex-sorted spermatozoa were of the correct sex
Centre for Advanced Technologies in Animal Genetics and Reproduction, The University of Sydney, Sydney	Sheep/Goat	51of the 55 (92.7%) lambs derived from fresh, sex-sorted frozen–thawed spermatozoa were of the predicted sex

Sperm Encapsulation

This technique Involves encapsulation of sperm for longer preservation of sperms *in- vivo* and allows progressive release of viable spermatozoa over several days in various domestic species including human. Various components used for encapsulation are, *viz*., calcium alginate, cellulose sulfate-poly-diallyl-dimethyl-ammonium chloride (CS-pDADMAC), poly-l-lysine, polyvinylamine and protamine sulfate membrane using standard encapsulation procedure. The size of the sperm capsule varies between 0.75 and 1.5 mm and sperm concentrations of 45-180 million/ml. Spermatozoa showed high motility rates within CS-pDADMAC based capsules as compared to other polymers, this technique prevents cryocapacitation and also reported to have increased conception rate.

Effect of Encapsulation on Sheep/Goat Sperms
After 8 days storage at 5°C, the viability and acrosome integrity of ram spermatozoa was lower for encapsulated spermatozoa than uncapsulated but, there was no difference between encapsulated and control semen regarding fertilisation rates (67%) (Maxwell *et al*. 1996) [1] Cohen *et al*., (2006) [2] recovered encapsulated spermatozoa with 96% efficiency in Buck

Transcriptomics
Transcriptomics involves the study of mRNA at various developmental stages including spermatogenesis, they are associated with different cellular and biological processes. Profiling of these transcripts provides highly effective tool for studying sperm mRNA expression profile analysis and polymorphism in related genes. Expression profile transcripts differ in high-fertility rams with higher concentrations of transcripts for membrane and extracellular space protein locations as compared to the low-fertility rams. Transcripts include protamine 1, casein beta 2 and thrombospondin receptor CD36 molecule. It is useful for elucidating transcriptomic changes associated with abnormal development in spermatogenesis and facilitates the improvement of assisted reproductive technologies and also serve as fertility markers (Biosennete *et al.*, 2009) [3].

Trancriptomics applied in Sheep/Goat
Mutations in the sheep BMPR-IB (FecB) gene has shown to be responsible on an increased ovulation rate and consequently larger litter size (Fabre *et al*., 2006) [4]. In goats, point mutation in the growth differentiation factor 9 (GDF9) gene, exerted a large effect on both ovulation rate and litter size (Niu *et al*., 2018) [5] The FGF5 gene, which is a dominant inhibitor of fiber length and growth has been successfully edited by CRISPR technology to promote their growth in offsprings (Hu *et al*., 2017).

Seminal Biomarkers
Proteomics and genomics have led to the identification of various biomarkers in various biological fluids including semen for predicting fertility. Some semen biomarkers associated with fertility in S/G are CATSPER family proteins, cysteine-rich secretory proteins, A-kinase anchor protein 4, cytochrome P450 aromatase, cathepsin D, α-l-fucosidase, spermadhesin Z13, clusterin, osteopontin, and PDC-109-like protein (Arangasamy *et al.*, 2005) [6] These proteins are critical for normal sperm motility and male fertility. The capability to identify males on the basis of these fertility markers could result in higher pregnancy rate, leading to increased crop (Srivastav *et al.*, 2003).

Ovum Pick Up (OPU)
This is a non-invasive and repeatable technique used for recovering large numbers of competent oocytes from antral follicles of live animals. Embryo production from ovum pick-up oocytes is affected by age, season, follicle stimulating hormone (FSH) stimulation and can average 1-3 embryos developed from oocytes collected per session. Repeated OPU can be performed without side effects. OPU has advantage to collect oocytes from animals with less invasiveness and the use of superior animals as oocyte donors in embryo transfer. Follicular aspiration also allows studying the molecular intricacy and the role of various cytokines during folliculogenesis (Prasad *et al.*, 2013) [8].

OPU – Technique application in Sheep/Goat
Results of an extensive research at Department of Animal Science, Faculty of Veterinary Medicine, University of Concepcio, Chile.

Table 2.

Procedure Applied	Success Rate	
	Sheep (%)	Goat (%)
Oocyte collection rate	85.32	81.65

***In Vitro* Maturation, Fertilization and Culture**
This involves oocyte collection from slaughterhouse ovaries or from live animals followed by maturation and fertilization *in vitro* for the production of viable embryos, IVMFC is an excellent source of embryos for embryo transfer, cloning and transgenesis. Technique has a 30-40% blastocyst development rate from oocytes after IVMFC

Efficiency of the technique
The process in Sheep/Goat is still inefficient:
Approximately 70–90% of immature oocytes undergo maturation, from prophase I to metaphase II; 50–80% undergo fertilization and cleave to at least the two-cell stage at 24 to 48 h post-insemination; Only 20% to 50% of immature oocytes ever reach the blastocyst stage, on day 7 to 8 post fertilization. It has also allowed the analysis of the developmental potential of embryos, pattern of gene expression, epigenetic modifications and cytogenetic disorders in various domestic species.

Intracytoplasmic Sperm Injection (ICSI)
ICSI is a micromanipulation technique used for treating male infertility. It involves mechanical insertion of a selected sperm into the cytoplasm of an oocyte to produce desirable embryo. ICSI has also been done with sexed semen with a success rate of 80% in cattle and 48-63% in small ruminants using fresh and frozen-thawed semen.

ICSI applied in Sheep/Goat
The mean rate of spontaneous fertilization after intracytoplasmic sperm injection was 32%, but 88% of the oocytes that failed to fertilize spontaneously did so after subsequent exposure to calcium ionophore Total of 71.8% of oocytes reached the 2-cell stage following living sperm injection. Cleavage rate reported 71% in goat and 50.7% in sheep by Wang *et al.*, 2006.

Embryo Transfer Technology (ETT)
ETT is an important tool to improve livestock at faster rate as well as provides an opportunity to utilize the genetic contribution of both male and female. ETT involves superovulation, an important step for increasing the number of oocyte from superior donors.
MOET programs could result in increased selection intensity and reduced generation intervals, resulting in increased genetic gain. Superovulation is carried out in donors using hormonal preparations mainly follicle stimulating hormones. On superovulation, there is increased in follicles and release of numerous ova from multiple follicles and hence double insemination with semen from superior bull. This is followed by flushing of embryos and transfer to recipient. Conception rate is around 35-45%. The first embryo transfer in sheep and goats.

Transferred 19, 2-16 cell embryos to 18 recipient ewes, eight lambs were born. The success of embryo transfer depends on management of donor and recipient animals, superovulation of donors, breeding (natural or artificial insemination), embryo collection and evaluation, transfer of embryo, and factors affecting survival of transferred embryos.

Recently, a goat ET group in Sri Lanka reported that they have produced ET born goat kids from fresh embryos in a field embryo transfer program without using sophisticated equipment, thus validating the field application of the technique in veterinary practice to multiply genetically valuable goats to establish an elite goat herd under local production conditions.

Embryo Cryopreservation

The mouse embryo was the first to be cryopreserved Vitrification was developed to counter the issue of low conception rates by cryopreserved embryos. This method involves the use of highly concentrated aqueous solution of cryoprotective agents, *viz*., glycerol, ethylene glycol, and non-permeating agents such as sucrose, glucose, and fructose. Successful for cryopreservation of Sheep and Goat embryos at various developmental stages with the recovery rate of 68-79%. Promising results are shown in open-pulled straw vitrification technique with embryo survival rate of 59 & 64% in Sheep and Goat respectively Reduces disease transmission and conservation of endangered species germplasm.

Embryo Sexing

Embryo sexing is a technique in reproductive biotechnology having practical applications. Procedures for embryo sexing have been used, *viz*., biopsy or cells aspiration. Embryos are collected on day 7 and are washed in buffer saline. Sex determination is performed by Y-chromosome-specific DNA probe technology coupled with polymerase chain reaction (PCR). The mean percentages of sex identification by embryo sexing at 2 cell, 4-8 cell, morula and blastocyst were 73.00±5.72, 89.77±3.79, 83.33±8.08 and 79.6±9.09% respectively in sheep. The biopsied samples from 51 goat embryos were amplified with 100% efficiency and 94.7% accuracy.

Somatic Cell Nuclear Transfer

Somatic cell nuclear transfer also termed as "cloning" involves utilization of micromanipulation technique and cell fusion to transfer blastomeres of multicellular embryo or somatic cell into enucleated oocytes, Ex- Dolly Sheep. Animal cloning was used for propagation of valuable genotypes, induce genetic modifications, and for producing transgenic. Technique can also be used in local breeds containing genes that confer adaptation, heat tolerance and disease resistance

Nanotechnology

In reproduction, microfluidics and nanofluidics are recent tools to simplify traditional procedures of IVF.

Oocyte manipulation under *in vitro* condition is also feasible with this technique. Microfluidics can be used in sorting of sperm and embryos. Development of biosensors for several markers has paved the way for identifying elite breeders. In farm animal, use of such sensors has been used in heat detection with nanotube under the skin to detect the changes in the level of estradiol in the blood. Biosensors for milk progesterone with detection limit between 0 and 5 ng/ml have been developed. Estrogen biosensor can detect to a limit of 10-150 pg/ml and the detection limits were 1-2 ng/ml and 1-1.8 ng/ml for FSH and LH.

Effect of elemental nano-selenium on semen quality, glutathione peroxidase activity, and testis ultrastructure in male Boer goats

Testicular Se level, semen glutathione peroxidase and ATPase activity increased significantly in the nano-Se supplementation group compared with control. The semen quality (volume, density, motility and pH) was not affected by added Se in diets. The sperm abnormality rate of control bucks was significantly higher than Se supplemented bucks.

Sequencing of endogenous beta-retroviruses of sheep

The sheep genome contains 27 JSRV-related endogenous betaretroviruses (enJSRVs) related to the pathogenic Jaagsiekte sheep retrovirus (JSRV). It was found that enJSRVs are able to protect the host against JSRV infection. In sheep, the enJSRVs are most abundantly expressed in the uterine epithelia.

Estrus synchronization protocols in Sheep/Goat

There are widely accepted protocols

1. 5 d CIDR insert(0.3 g P_4)
2. 5 d CIDR and PG (Lutalyse, 10 mg dinoprosttromethaminei.m., at CIDR removal
3. GnRH (0.025 mg gonadorelin hydrochloride i.m)at CIDR insertion and PG at CIDR removal

Challenges and Future Prospective

Database on indigenous livestock and its biodiversity including production, reproduction, disease resistant traits within species and breeds necessary for the implementation of these techniques. The use of these advanced techniques can further provide insight to the molecular intricacies of reproductive process including its derangement, in future. These emerging techniques should be judicially supplemented with good practices in animal health, nutrition and management for manipulation and improvement of health, production and reproductive performance.

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