Review

Assisted reproductive techniques in farm animals

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ABSTRACT

Animal productivity is highly dependent on reproduction, which is the key to growth. Reproductive inefficiency is a well accepted factor contributing significantly to financial losses in the animal industries. Despite the impressive progress made in the science of reproductive physiology recently, infertility caused by low conception rates and high embryonic mortality rates continues to be a significant issue. It is necessary to use all new technologies, particularly contemporary reproductive biotechnologies, to support agricultural production, agricultural research, and its uses in order to meet present and future demands. Due to the lack of affordable embryos from high-quality animals, development of reproductive methods such as super ovulation, estrus synchronisation, non-surgical embryo transfer, oocyte collection from live animals, in vitro fertilisation, embryo development and cloning could not have an impact on the production of excellent animals. The goal of the current review was to increase understanding of the different modern assisted reproductive procedures that could help to improve the state of animal reproduction today.

Keywords: Farm animals; reproductive techniques; embryos; reproduction

INTRODUCTION

Productivity is the key to growth and reproduction is the backbone of animal production. Reproductive inefficiency is one of the most important causes of economic losses in animal industries and it is realized throughout the world. Despite of remarkable advancement that has been made in the field of reproductive physiology in recent years, infertility due to low conception rate and high embryonic mortality rate remains a major problem. To meet future needs and to be able to sustain agricultural production, agricultural research and its applications, there is a need to use all emerging technologies especially the modern reproductive biotechnologies. Development of reproductive techniques like estrus synchronization, super ovulation, non-surgical embryo collection, transfer, cryopreservation of embryos, oocytes pick-up from live animals, in vitro maturation, fertilization, embryo development and cloning could not make an impact on quality animal production due to non-availability of low cost embryos from quality animals (Verma et al 2012). In the present review, efforts have been made to enrich the knowledge about various recent assisted reproductive techniques which may be helpful for improving the current status of livestock reproduction.

Artificial insemination (AI)

Developed and developing nations alike are currently using this technology in their commercial dairy

cow programmes. Through the global distribution of semen, the frozen semen (Polge et al 1949) revolutionised the AI programme. According to Boa-Amponsem and Minozzi (2006), AI technology increases the use of exceptional males, spreads superior genetic material, increases the rate and efficacy of genetic selection, introduces new genetic material by importing frozen semen rather than live animals and thus lowers the cost of international transportation, makes it possible to use frozen semen even after the male has passed away and lowers the hazards of sexually transmitted diseases spreading.

Regarding the current state of artificial insemination in India, 44 million frozen semen straws were produced and 41 million AI with a conception rate of 35 per cent were produced in 2008 (Anon 2008). Because field AI operations in developing nations have a relatively low conception rate, the predicted improvement in animal quality has not been attained. Technical incompetence and improper management are to blame. AI won't become more efficient until farmers have access to vastly improved organisational and technical infrastructure (Anon 2020).

IVM/IVF embryo production

In vitro fertilisation has the ability to produce genetically better embryos on a commercial scale. With the characterisation of successfully defined and semi-defined media for various species, the rate of progress has accelerated in the recent decades; there has been an unparalleled evolution of in vitro embryo production (IVEP) technology in farm animals. Technologies used in in vitro production not only aid in the creation of animals with high genetic merit, but also serve as a good source of embryos for cutting-edge biotechnologies like nuclear sexing, cloning and embryo sexing, which greatly reduce these challenges. Oocytes can be aspirated from living animals using ovum pick-up (OPU). Currently, OPU-IVEP is employed in several nations for commercial level and large scale embryo production. Thanks to this repeated recovery that more embryos can be produced than might otherwise be possible using regular embryo transfer (ET) procedures (Galli and Lazzari 2008).

Intra-cytoplasmic sperm injection (ICSI)

Intra-cytoplasmic sperm injection was developed as a successful treatment for male infertility of various causes and this sparked renewed interest in its possible application to farm animal reproduction. ICSI can be used to produce transgenic animals and explore the mechanism of fertilisation in addition to its clinical applications. ICSI is a method of micro-fertilization in which a single spermatozoon or sperm head is directly injected into the ooplasm. The primary reason for ICSI is severe male infertility brought on by a variety of disorders of testicles, epididymis or ejaculated spermatozoa. ICSI has been used on domestic species with varying degrees of effectiveness, including cattle (Horiuchi et al 2002), pigs (Martin 2000) and small ruminants (Catt et al 1996). IVF in horses has shown to be a highly challenging and problematic technique. Thus for this species, ICSI approach has been favoured (Colleoni et al 2007).

Sexing of semen and embryos

Predicting the gender of the offspring would result in more males or females, allowing for the selection of individuals with the best genetic make-up for the advancement of the following generation (Plummer and Beckett 2006). Since more heifer calves may be produced per embryo transfer (ET) when using known sex embryos, managing producer resources can be done more effectively. The presence or absence of components typically found on the Y chromosome determines whether an embryo is sexually differentiated. Commercial methods for embryo sexing include chromosomal examination of diploid embryos, immunological identification of embryonic H-Y antigen, use of Y-specific probes, fluorescence in situ hybridization, using the loop-mediated isothermal amplification (LAMP) response etc and a quick sexing approach for bovine pre-implantation embryos has been developed (Zoheir and Allam 2010).

Another method involves separating semen into male and female components one sperm at a time using a staining approach and laser beam detection with the aid of conventional flow cytometry equipment (Garner 2006).

Therefore, female or male embryos could be created using sperm with an X or Y chromosome. The buffalo, Indian zebu and Taurus cattle share the same Y-chromosome-specific sequences (Rao et al 1993). Therefore, embryonic sexuality of buffalo or Indian zebu cattle can be determined by using primers that are specific to the bovine Y chromosome. Additionally, an effective embryo biopsy technique has been established (Lopatarova et al 2008). According to recent developments in semen sexing, successful production of pre-determined sex offspring (Garner 2006) in

several mammalian species, including cattle (Seidel et al 1999), goats (Parrilla et al 2004), pigs (Grossfeld et al 2005) and sheep (de Graaf et al 2007), using fresh and frozen-thawed spermatozoa, has been done. Aside from China, none of the emerging nations have documented any cases of field reports of embryo and sperm sex.

Oocyte/embryo cryopreservation

Due of the relatively brief productive life of mammalian oocytes, continuous supply of viable, developmentally competent oocytes has been essential to recent advancements in IVEP. Therefore, storing unfertilized oocytes would result in a conveniently accessible supply, allowing studies to be conducted at a suitable moment. This might be useful in the creation of a gamete bank from which certain genetic combinations could be created. Significant advancements have been made in the cryopreservation of mammalian oocytes and embryos over the past few decades. Transfer of cryopreserved embryos or oocytes resulted in live progeny of at least 25 species (Gajda and Smorg 2009). Oocyte preservation lowers the cost and risk of transporting live animals as well as the dangers of disease transmission. Additionally, it provides protection from catastrophes and natural disasters. The oocyte preservation in threatened species protects them from extinction.

Damage caused by mechanical and osmotic forces throughout processing steps is the main issue in cryopreserving germplasm. Glycerol's use as a cryoprotecting agent made cryopreservation processes (CPs) more practical. Many people utilise other CPs as well, either alone or in different combinations. These include penetrating CPs like ethylene glycol (EG) and propylene glycol as well as non-permeating sugars like sucrose, glucose or fructose (Barcelo-Fimbres and Seidel 2007). The development of conventional cryopreservation techniques led to the introduction of vitrification of germplasm (Rall and Fahy 1985). Transfer of cryopreserved embryos or oocytes resulted in live progeny of at least 25 species (Gajda and Smorg 2009).

Oocyte preservation in threatened species protects against extinction. Mechanical and osmotic damage that occurs throughout processing steps is the main issue in cryopreserving germplasm. The development of glycerol as a cryoprotecting agent made cryopreservation more practical. Numerous more CPs is also in use, either separately or in other combinations. These include penetrating CPs such as ethylene glycol (EG) and propylene glycol (Luz et al 2009) as well as non-permeating CPs like sucrose, glucose or fructose (Barcelo-Fimbres and Seidel 2007).

Vitrification of germplasm was introduced with the advancement of conventional cryopreservation techniques (Rall and Fahy 1985). In comparison to slow freezing, vitrification is an easy, quick and less expensive process. Bovine (Hochi et al 2000), swine (Huang and Holtz 2002), horse (Hurt et al 2000) and buffalo (Sharma and Loganathasamy 2006) oocytes have all been subjected to vitrification-based cryopreservation with varying degrees of success. Cryobanking of embryos for endangered species may be useful in creating founder populations in preparation for potential reintroduction into the wild (Park et al 2002).

Embryo transfer

The use of embryo transfer technology is essential for increasing the rate of livestock improvement and for exploiting both the genetic contributions of male and female cattle at the same time. ET (embryo transfer) and MOET (multiple ovulation embryo transfer) techniques can be used to enhance livestock more quickly, increase the number of elite animals quickly, acquire genetic advantage, hasten the development of herds and preserve unusual genetic stocks (Nicholas and Smith 1983). Embryo transfer and other related procedures were carried out on a global scale on 25,000 sheep, 7,000 goats, 30,000 pigs and 12,000 horses (two-third of which were derived from in vivo sources and the other third from in vitro sources), with a conception rate of 55-70 per cent (Thibier 2009).

Embryo genomics

A few of the most recent biological approaches being developed are quantitative real-time PCR (RT-PCR), subtractive cDNA libraries and differential display reverse transcription-polymerase chain reaction (DDRT-PCR) that will greatly expand our ability to identify variations in mRNA expression patterns during pre-implantation development. In order to choose markers for identifying high quality embryos, it will be helpful to know how various genes express themselves during pre-implantation development. In order to develop reproductive technology and evaluate the embryos' health, this information may also be utilised. In bovine and murine embryos in the pre-implantation stage, the expression pattern of numerous classes of genes has been examined using qualitative and quantitative RT-

PCR tests (Niemann and Wrenzycki 2000). In murine embryos, it encompassed more than 32 physiological activities including the expression of over 250 different genes while in farm animal embryos, it covered around 60 to 70 genes and its physiological functions (Wrenzycki et al 2005).

Cloning

Cloning is an effective approach that may be used in experimental animals to enhance the number of elite animals and decrease genetic variation. It can be used to preserve endangered species and to spread them. It could be a method for producing stem cells for medical treatment or for therapeutic cloning. Through the use of somatic cells, it is possible to choose and reproduce animals with particular qualities (Das et al 2003). In somatic cloning, donors can include foetal fibroblasts, adult fibroblasts, granulosa cells, hepatocytes, lymphocytes and a variety of other somatic cell types (Campbell et al 2007). Sheep, named Dolly, was the first animal created using somatic cloning (Wilmut et al 1997).

Nuclear transfer-derived embryonic stem cell (NTESC) refers to the cloning process that uses embryonic stem cells (ESCs). Farm animal embryonic stem cells (faESCs), despite generated from humans and some laboratory animals, have not yet been successful (Beyhan et al 2007). EGCs and spermatogonia stem cells may be used as a substitute for faESCs (Brevini et al 2008).

Cloning could be utilised in xenotransplantation in the future to produce more humanised pigs whose organs could be used to transplant into humans (Duszewska and Reklewski 2007). In a survey on the use of cloning technology done in 2005 by the OIE (MacKenzie 2006), 4 per cent of respondents were from Africa and 23 per cent were from developing nations.

A female cloned camel named Injaz and a second male cloned camel named Bin Soughan were recently added to the list of cloned animals. They were both born at the Camel Reproduction Centre in Dubai, United Arab Emirates. At NDRI, Karnal, India, the world's first buffalo calf, GARIMA-I (2009), was born; thanks to the introduction of a novel technology called hand guided cloning technique. Later, using the same method, the same institute developed the male buffalo calf Shresth (2010) and GARIMA-II (2009).

Transgenesis

Genetic engineering has completely changed all facets of basic biology and medicinal research since the original demonstration of the feasibility of producing transgenic animals in the 1980s. Transgenic animals are currently produced using a variety of biotechnological techniques, including pro-nuclear micro-injection, cytoplasmic micro-injection, retrovirus-based vectors, DNA transfer via retroviral vectors to embryos or embryonic stem cells, sperm-mediated gene transfer of lenti vectors and RNA interference (Robl et al 2007). Both breeding and biomedical applications are possible for transgenic farm animals (Wells 2010).

Transgenic individuals are created during breeding and come with enhanced quantitative and qualitative features as well as disease resistance. For example, transgenic sheep with an integrated keratin-IGF-I gene have higher wool production and sheep and goats have antitrombin III and **α**-antitrypsin in their milk (Kues and Niemann 2004).

Transgenic pigs with high body weight gain or a high ratio of fat to muscle tissue also express human growth hormone and human haemoglobin (Kues and Niemann 2004). The creation of transgenic cows with mastitis resistance was a significant accomplishment (Wall et al 2005). The creation of environmental friendly transgenic people is the subject of ongoing research, as is the use of such animals in fundamental studies to better understand the different physiological processes that occur in both farm animals and people (Niemann et al 2005).

Stem cell technology

In all multicellular creatures, stem cells are present. They can differentiate into a wide variety of specialised cell types and still maintain the capacity for self-renewal through mitotic cell division. According to Bajada et al (2008), stem cells are used as a model for developmental biology in organ transplantation, gene therapy, medication development, creation of chimaeras and in the field of regenerative medicine. In terms of its use in farm animal reproduction, embryonic stem cells are crucial because they give researchers a way to precisely modify animals' genetic makeup, either through somatic cell nuclear transfer or homologous recombination of embryonic stem (ES) cells (Lombardo et al 2007). Providing large animal models in which the ES cell technique may be explored for tissue-specific

differentiation and cell treatment of diverse tissues and organs is a second significant application (Brown et al 2007).

In rodents, the transfer of spermatogonial stem cells (SSCs) is frequently utilised to examine the regulation of spermatogenesis with the ultimate aim of enhancing or suppressing male sterility. Cattle can easily adapt successfully transferring SSCs from goat and pig (Honaramooz et al 2003).

In commercial breeding systems for cattle, germ line transfer has potential application. Elite genetics could be spread more extensively by implanting SSCs from superior bulls into inferior males and then allowing for natural service (Herrid et al 2006). In locations, where artificial insemination is not feasible, this technique might offer a substitute for it for the use of exceptional sires in the cattle business (Hill and Dobrinski 2006). Herrid et al (2006) showed that effective male germ cell transplantation across different cattle breeds and unrelated bull calves was also possible. When it comes to their prospective uses in many scientific fields and farm animal reproduction, the current understanding of the biology of stem cells is limited, but research is ongoing to fill in the gaps.

Nanotechnology

The field of cellular and molecular biotechnology has recently made advances in nanotechnology. In addition to its uses in genetics, biotechnology, therapeutic medicine and cellular biology, it may also be a valuable tool in farm animal breeding and reproduction. The mobility and handling of an embryo in a microfluidic environment was first demonstrated by Glasgow et al (2001). The separation of sperm and eggs is another application for it. The flow of liquids or gases is controlled by the systems through a series of micro- and nano-scale channels and valves. A computer circuit aids in the data analysis process. With the help of nanotechnology, genome mapping and sequencing, it is possible to uncover gene sequences linked to features that are desirable commercially, like as disease resistance and meat leanness. Breeders will be able to quickly identify exceptional breeders and screen out genetic disorders by inserting probes for these qualities on biochips. By subcutaneous implantation of a nanotube to monitor variations in the blood's level of estradiol, heat detection in farm animal breeding can be accomplished (O'Connell et al 2002).

CONCLUSION

Following the invention of artificial insemination in the 1980s, recent advancements in assisted reproduction in farm animals has picked up interest again. A significant factor in this growing interest is the desire to create cloned and transgenic founder animals that can produce chemicals beneficial to the pharmaceutical industry. A useful technique for determining the causes of numerous problems at the cellular level is embryo genomics, such as early embryonic mortality brought on by improper gene expression. The science of assisted reproduction has advanced, thanks to the discovery of stem cells and nanotechnology opening the door to the creation of animals with the necessary traits. These ongoing initiatives will have positive long term effects on the global farm animal industry as a whole.

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