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Recent trends in bovine reproductive biotechnologies

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Abstract

The establishment of non-invasive and low-cost reproductive techniques are key interest currently. Although, numerous reproductive techniques have been invented during the last few decades, the procedures adopted require further refinement. Artificial insemination is a unique and simple reproductive technique offering substantial and rapid genetic transfer. Use of fresh semen has given good results, but success and reach of frozen semen technology is controversial in developing countries. Optimal use of sexed semen requires precise accuracy in ovulation to obtain satisfactory pregnancy rates. Further modification of the sperm sorter technique is necessary to obtain the maximum number of sexed sperm within a limited time. Embryo transfer technology has been well developed for dairy animals, but currently the main focus is on *in-vitro* embryo production. Its importance is further magnified for animals with good genetics and species near to extinction. Although cloning has been successfully carried out in many species, its cost, neonatal abnormalities and ethical use require further discussion. Transgenesis and stem cells have revolutionized the world through the production of transgenic animals and disease management through mesenchymal stem cells. These technologies are the advanced reproductive technologies which need further research, especially in bovines. In conclusion, modern reproductive techniques are a basic requirement of a modern dairy farming system but their use must be rationalized in an ethical and cost-effective manner.

Keywords: *In-vitro* embryo production, Embryo transfer, Artificial insemination, Cloning

Review Methodology: We searched the resources of the International Embryo Transfer Society. In addition, we used the references from the articles to check for additional relevant material. We also spoke to the colleagues working on the *in vitro* embryo production to know about their experiences and new studies they are conducting.

Artificial Insemination

Artificial insemination (AI) is a technology in which sperm are introduced into the reproductive tract of the female by means other than sexual intercourse. Arabic countries were the first to introduce the concept of AI. They took semen by swabbing the vagina of a mare recently bred to a stallion of high-genetic quality and then inserted the swab into the vagina of another mare that was in heat and the mare became pregnant. However, Spallanzani performed the first documented AI in dogs [1]. AI in domestic animals was practically started at a large scale by the Russian scientist Ivanoff [2]. AI has been widely used in advanced countries, but the coverage of AI is more limited in developing countries. According to a survey conducted during 1991, the percentages of developing countries not producing semen and producing semen were 22.1 and 53.9%, respectively [3]. According to a survey conducted in 1998, 50 and 27% of the total semen production is by Europe and by the Far East, respectively. However, 50% of total inseminations were performed in Far East, while only 34% inseminations were performed in Europe [4]. AI coverage rates in the developing countries are quite low, with just 35 and 14% in India and Pakistan, respectively [5]. AI has been reported as first reproductive biotechnology for improving the reproductive efficiency and genetics of

Table 1 Propagation of different reproductive techniques from animalcules to Dolly

Author	Publication journal	Year	Innovation
van Leeuwenhoek	*Philosophical Transactions*	1677	Animalcules (sperm cells)
Stensen	–	1667	'Female testes' contained oocytes
De Graaf	*De clijsteribus et de usu siphonis in anatomia*	1668	Graafian follicles
Spallanzani	*Dissertations Relative to the Natural History of Animals and Vegetables*	1784	First successful insemination in dog
Hunter	*An anatomical description of the human gravid uterus, and its contents*	1794	Corpora lutea associated with pregnancy
Von Baer	*De ovimammalium et homonisgenesi*	1826	Identified the mammalian oocyte
Heape	*Proceedings of the Royal Society London B*	1897	Insemination in rabbits, dogs and horses
Ivanow	*Journal of Agricultural Science*	1922	Insemination in domestic farm animals
Phillips and Lardy	*Journal of Dairy Science*	1940	Yolk-phosphate semen extender
Sørensen	*Danske Dyrlaegeforen*	1940	Straw for packaging semen
Salisbury *et al*.	*Journal of Dairy Science*	1941	Buffering the egg yolk with sodium citrate
Trimberger	*Nebraska Agricultural Experiment Station Bulletin, Lincoln*	1948	A.M. to P.M. rule for AI
Polge *et al*.	*Nature*	1949	Cryoprotective properties of glycerol by
Foote and Bratton	*Journal of Dairy Science*	1949	Extender containing the antibiotic
Willett *et al*.	*Science*	1951	First successful transfer of fertilized bovine ovum
Morrow	*Journal of Dairy Science*	1968	Reproductive herd health programme
Pierson and Ginther	*Theriogenology*	1984	Use of ultrasonography to measure follicle diameter
Ginther	*Theriogenology*	1984	Ultrasonic anatomy and pathology of uterus
Gledhill	*Gamete Research*	1985	Sexing of sperm by DNA quantification
Rant	*Animal Reproduction Science*	1986	Use of ultrasound in bovines
Hopkins and Evans	*Veterinary Endocrinology and Reproduction*	1989	Buffer in extender
Wilmut *et al*.	*Nature*	1996	Dolly cloned

animals. AI remains at the top in the list of assisted reproductive technologies (ART) despite remarkable progress made in all other reproductive biotechnologies. ART [6] include all fertility treatments in which both eggs and embryos are handled. In general, ART procedures involve removing eggs from ovaries, combining them with sperm in a laboratory and returning them to the uterus. To be used in genetic exploitation, any new reproductive biotechnology should compete with AI in terms of simplicity and success rate as a reproductive tool, given the predominant economic conditions in growing countries. AI is being used widely in dairy reproduction systems to uplift the efficacy of transfer of genetic material in commercial sectors [7]. The biggest challenge for a new reproductive biotechnology to gain popularity is to resemble AI which is economical, successful and simple [8]. Through extended semen, the genetic worth of a bull can be increased up to 50% by using AI [9]. AI has been widely used in many domestic animals over recent decades but further refinement is required in extender preparation, semen processing, semen thawing and semen deposition. Various antibiotics in single [10] or combined form [11] have been tried to minimize the load of bacteria without any negative effect on the post-thaw semen quality. Various plant components such as lecithin from soya bean [12], and extracts such as *Camellia sinensis* and *Syzygium aromaticum* improved the semen quality during cryopreservation and decreased motility and mitochondrial activity during liquid storage and at the time of thawing of semen [13]. After the pasteurization process, egg yolk in powder form could be a safer alternative [14, 15]. A lower fertility rate has been observed in buffalo as compared with cattle after insemination due to poor response of sperms to undergoing the process of cryopreservation in buffalo bulls [16]. Positive effect of fish oil on the post thaw semen quality has been observed in buffalo [17] (Tables 1 and 2).

Table 2 AI coverage rate in developing countries (buffalo)

Country	Total population (million)	AI coverage (%)
Pakistan	38.0	14
India	108	35
China	23.0	N/A
Italy	0.26	5
Azerbaijan	3.00	3.7
Egypt	3.17	0.3
Romania	1.00	0.1
Philippines	3.20	N/A
Thailand	1.70	N/A
Malaysia	0.17	0

Data are reported from Buffalo reproduction and Research by Antonio Borghese.

Genomic selection

Low milk production is one of the problems that local livestock is facing along with the lack of superior bulls for

improvement in future generations. Identification of superior bulls for increasing milk production is possible through selection by estimation of breeding values and genome analysis. Genomic selection refers to selection decisions based on genomic breeding values [18]. The genetic gain could be doubled through genomic selection [19]. Genomic selection involves two developments. The first development was the recent sequencing of the bovine genome, which led to the discovery of many thousands of DNA markers. The second development was the demonstration that it was possible to make very accurate selection decisions through the estimation of breeding values from DNA markers [20]. Genotyping by sequencing (GBS) technique is a novel, flexible, sufficiently high-throughput and capable of providing acceptable marker density for genomic selection or genome-wide association studies. GBS is one of the most commonly used genome analysis methods.

Gene editing

The intended alteration of a desired DNA sequence in a living cell is called genome editing. The techniques used for gene editing involve the usage of certain proteins for cutting of targeted DNA in a precise manner. CRISPR-Cas9 is a widely used genome editing method. CRISPR stands for 'clustered regularly interspaced short palindromic repeats'. The ease, expedience and efficiency of the CRISPR/Cas9 system have enabled its use in a variety of applications, including genome editing, gene function investigation and gene therapy in animals and human cells [21]. The gene editing in bulls will help in preventing the inheritance of disease traits through AI.

Sperm Sexing

The demand for sexed semen has been increased dramatically from the last decade. Sperms of interest are sorted using a flow cytometer with the speed of 15 million spermatozoa per hour [22]. A minimum of 4 million spermatozoa represents the optimal compromise pregnancy in Mediterranean buffaloes, compared with conventional technologies of insemination together with oestrus synchronization [23]. A conception rate of 30–50% has been observed in Mediterranean buffalo after insemination with sexed semen. The conception rate in buffalo heifers was also better with sexed semen compared with conventional semen [24]. One of the special applications of sexed semen is for *in vitro* fertilization (IVF) by decreasing the cost and logistics of embryo transfer (ET) [25]. Lower sexed sperm numbers are not a constraint for a lower pregnancy rate and now researchers are focusing on the efforts for decreasing the cost of production and prevention of sperm injury during sorting and freezing. Recent development in sperm sexing include the use of two or more nozzles in the configuration of sperm sorter instead of one, which will speed up the sperm sexing process and decrease cost of production. Insemination in lactating Jersey cows near ovulation yielded higher pregnancy rates [26]. After synchronization and fixed time artificial insemination (FTAI), similar conception rates have been observed in beef cattle and heifers [27] by the use of conventional and sexed semen. Similar pregnancy rates have been observed in mature beef cows by using sexed semen and conventional semen after FTAI and split time AI [28]. However, decreased levels of transferable embryos have been reported after using sexed semen compared with non-sexed semen in super-ovulated dairy cows [29]. An increase in the pregnancy rate was observed through AI by using sexed semen in heifers of buffalo when compared with conventional semen [30]. Conception rate can be further increased if the sexed semen is deposited near the junction of uterus and oviduct by using a special catheter [31]. The pregnancy rate was non-significant in heifers and in buffalo that has parturated at least once and cows after using sexed semen [24]. Half dose of sexed semen gave 94% more results compared with non-sexed semen in lactating dairy cattle [32]. Liquid sexed semen generated more replacement heifers as compared with frozen-thawed semen and its negative effect on herd fertility reduced the farm profitability [33]. In heifers the use of fresh sexed semen facilitated more profit and faster genetic expansion as compared with the use of frozen-thawed and conventional semen [33]. However, high calf mortality and low ET rates have been observed in cattle after using sexed compared with conventional semen [29]. Lower conception rate has been observed in heifers and cows after using sexed compared with conventional semen. However, difficult births decreased up to 28% in cows after using sexed semen.

Multiple Ovulations and Embryo Transfer (MOET)

MOET is most common *in-vivo* reproductive biotechnology for the embryo production. The birth of first calf through ET technology involved the surgical transfer of 5th day embryos derived from abattoir-based ovaries in 1951 in Wisconsin [34]. Different protocols for superovulation of cattle and buffalo were established in 1987 [35]. Surgical and non-surgical techniques are used for flushing of embryos but the non-surgical technique is mostly used in bovines. The recovery rate of embryos from the surgical method is higher compared with the non-surgical method but it is more laborious and time consuming [36]. MOET is now well developed and commercially used across the world. According to the 2016 statistics for embryo collection and transfer in domestic animals by the International Embryo Transfer Society, the contribution of North America, Europe and Asia to the global embryo production was 52, 20 and 17%, respectively, while the percentages of global embryos transferred in

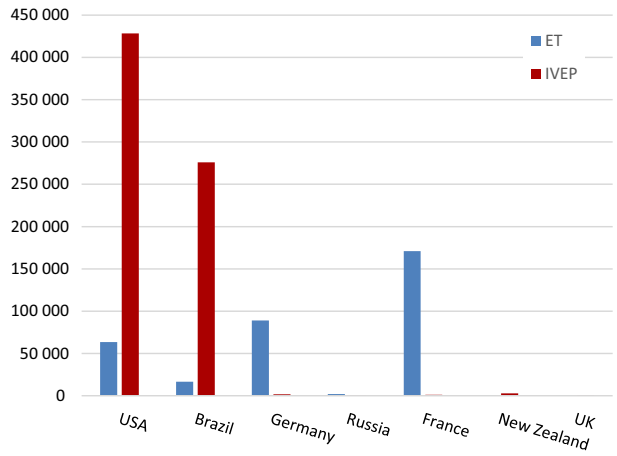

Figure 1 Contribution of different reproductive techniques for uplifting the dairy sector in developed countries (data reported by George Perry, 2018).

North America, Europe and Asia were 52, 22 and 14%, respectively. The average number of ET calves produced from donors has increased from 1 to more than 10 per year [37]. More than 750 000 embryos, on average, are being produced from superovulated animals [38]. One of the best uses of MOET is to produce progeny tested sires from proven cows and bulls [39]. In buffalo and cattle the conception rate following ET is 35–45% [40]. ET technology has been used in buffaloes but the results are low and progress is modest to poor [41]. However, this technology can increase significantly the chance of inbreeding, owing to the limited number of elite dams and short intervals can lead to more chances of inbreeding [42] (Figure 1). Developmental competence of buffalo embryos is faster as compared with cows [43, 44]. The higher growth rate in embryos attained through cross breeding compared with embryos produced by pure breeding suggesting the use of cross breeding in reproductive biotechnologies [45]. For ET, synchronization of donor and recipient is a prerequisite for proper embryo recovery, ET and recognition of embryo in recipient cattle [46]. Embryo sexing by DNA having Y chromosome probe attached to polymerase chain reaction (PCR), loop-mediated isothermal multiplication, detection of H-Y antigen, duplex PCR-based assay is >95% accurate [47, 48].

In Vitro Embryo Production (IVEP)

This modern reproductive biotechnology includes harvesting of the oocytes by aspiration, *in vitro* maturation (IVM), fertilization and *in vitro* culture (IVC). The number of embryos produced through IVEP can be two–three times more than MOET within a given period of time [42] (Figure 1). This technique is more flexible as compared with MOET, because in this technology oocytes can be retrieved by the aspiration of slaughterhouse-derived ovaries and by ovum pickup technology from the live animals. According to the 2016 statistics of embryo collection and transfer in domestic animals from the International Embryo Transfer Society (IETS), the highest number of slaughterhouse-derived embryos were produced by South America and Europe (1511 and 1095 respectively). However, the number of fresh and frozen embryos transferred in South America and Europe during 2016 were only 0 and 173, respectively. There was no data of IVEP in Asia during 2016. Ovum pick up (OPU) is better than superovulation because it yields better transferable embryos per donor on monthly basis. According to IETS evidence for 2016, a total of 666 215 OPU-IVF-derived embryos were produced during 2016, out of which the highest embryo production was by South America, producing 378 291 while the lowest production was by Asia, which was 0. Similarly, the number of OPU-IVF-derived embryos transferred were highest in South America, at almost 30 000. However, the transfer rate of OPU-IVF-derived embryos in Asia during 2016 was zero. Juvenile ET is an emerging technology based on IVM, fertilization and ET to reduce the generation interval [49]. In this technology, OPU is conducted in female calves ranging over 2–3 months of age through laparotomy and processed for IVM, fertilization and transfer of embryos after growing to the stage of blastocyst [50]. In 1981, the first calf was produced through IVF [51]. IVF is a basic technique which leads to the advanced reproductive biotechnologies including cloning and microinjection [52]. Using this technology, more than 450 000 embryos have been produced. The success of blastocyst from ovum following IVM, IVF and culture is 30–40% [53]. The low success rate and high cost of production made this technique less feasible in buffalo and other livestock species under field conditions [41].

In vitro *maturation (IVM)*

Various media are available for IVM of oocyte and the most used media are tissue culture medium 199 and Ham's F-10a. The preference of media needed for maturation is different in various laboratories. Maturation media may be supplemented with foetal calf serum, oviduct synthetic fluid, bovine serum albumin (BSA), newborn calf serum [54], hormonal preparations such as follicle-stimulating hormone, equine chorionic gonadotropin, human chorionic gonadotropin and various growth factors for example insulin, insulin-like, epidermal plus fibroblast growth factor and transferrin sodium selenite [55]. Supplementation of maturation media with such natural and synthetic products is different according to the requirements of labs.

In vitro *fertilization (IVF)*

For fertilization, approximately 10 000 spermatozoa are incubated with an oocyte. Fertilization is an important

step in the IVEP, because preparation of spermatozoa and inducing the artificial capacitation is necessary. Capacitation is carried out by heparin-like glycosaminoglycan present in the oviductal fluid. The capacitation of spermatozoa in bovines is mostly done using Tyrode Albumin-Lactate-Pyruvate [56]. However, Brackett and Oliphant medium is used in some studies. The addition of heparin [57], media with high-ionic strength [51], epinephrine and hypotaurine, caffeine and calcium ionophores gives higher fertilization rates. Fertilization rate after IVF has been reported to be above 80% in cattle.

Methods for sperm capacitation

Swim-up method

Mahadevan in 1984 introduced the technique of swim-up for the capacitation of spermatozoa. There is widespread use of this technique around the world, which gives good cleavage rates and is still a well-adapted technique for the IVF and intrauterine insemination [58]. This technique is low cost and very simple, and does not require highly specialized expertise or sophisticated equipment. Perish [56] described a new swim-up technique which uses two types of media separately: Tyrode albumin-lactate-pyruvate and modified Ca^{2+} free Tyrode's medium. The major advantage of this technique is that it does not require any costly media or sophistication and higher cleavage rate is achieved because of less trauma to sperm [59]. However, the concentration of sperm is compromised in this technique.

Swim-down method

The basis of this technique lies in the natural movement of sperms. A discontinuous medium containing BSA is prepared. The concentration of this medium decreases progressively from top to bottom. The semen sample is placed on the upper surface of the medium and the incubation of tube containing media is at 37 °C for 1 h [60]. During migratory movement, the sperm with highest motility move downwards into the gradient. Through the swim-down procedure a higher recovery rate and sperms with higher progressive motility can be separated [61].

Percoll density gradient

Percoll density gradient is also a good technique used for the capacitation of the spermatozoa. In this technique, media with different densities are used to capacitate the sperms. Owing to the quicker penetration than swim-up, the highly progressive motile sperm reach the bottom of the centrifuge tube faster than immotile and poorly motile sperms, which remained at the boundaries of interphases [62]. The major advantage of this technique is that it provides a higher concentration of sperms after capacitation. On the negative side, Percoll is a toxic agent which has endotoxic effects on the sperm [63], but currently this technique is modified with the washing of sperm after the capacitation with capacitation media to minimize the toxic effect of Percoll.

In vitro *culture (IVC)*

Co-culture of zygotes with various types of amino acids, cells and synthetic products gave excellent results. Secretions of female reproductive tract contain amino acids and could be used as a substrate for energy by embryos [6]. Embryo development improved after using the amino acid in serum-free media for culture [64]. Cell fragmentation during the IVC can be reduced by the use of amino acids [65]. Synthetic Oviductal Fluid (SOF) is most widely used chemically defined medium by various labs [66]. These media (SOF, SOF + BSA) improved the blastocyst rate up to 20% at day 8. Various types of cells such as cumulus cells, established cell lines, epithelial cells from bovine oviduct [67] and liver cells from the buffalo have been used for culturing of embryos.

Intracytoplasmic Sperm Injection (ICSI)

Lin in 1966 reported this injection procedure for the first time in mammalian egg [68]. ICSI, as its name indicates, is the penetration of a sperm into a matured ooplasm at metaphase II stage with the help of a microscope and needle. ICSI is a powerful tool and provides new directions to study basic concept of fertility and early embryonic development. Zygote formation from the ICSI led to declining in the number of blastocyst formation and less live births of calves. Therefore, ICSI is a technique of low efficiency as compared with other reproductive biotechnologies such as IVEP [69]. Spermatozoa used for ICSI don't need to be motile and their tails must be broken before injection [70]. To achieve fertilization and development, independent of sperm collection, characteristics and storage, the ICSI is a powerful micromanipulation tool [71]. Low blastocyst production rates and high abnormal fertilization rates after ICSI have been observed compared with IVF [72]. Injection of immotile and round-headed sperm decreased the cleavage rate but the quality of embryos was not affected [73]. This means that the centrosome plays an important role in first mitotic division but not in meiosis [74]. A better cleavage rate was reported after treating the oocyte with the calcium ionophores before ICSI and after ICSI [75, 76]. The improvement in the survival rate of oocytes was observed after treatment with K^+-rich or Na^+-deficient media for culture in ICSI [77].

Cloning

Cloning is the process of creating identical copies of organism by asexual means. After the invention of Dolly

the sheep in 1997 from adult stem cell, cloning was banned in many countries on a legal and ethical basis, especially human cloning. However, a total of 22 animals of different species have been produced by cloning including cattle [78].

Embryo splitting

Embryos show totipotency until the 8-cell stage. A single cell of this embryo gives rise to the birth of new offspring. Owing to the totipotency of early embryonic cells [79, 80] the chances of splitting the blastomere from embryos at the stage of cleavage are improved, resulting an increase in the number of available embryos. The most effective and fast method to increase the number of offspring is always embryo splitting. The term cloning has also been used for this technology, owing to the splitting of embryo into two or more embryos [81, 82]. Compared with cloning there is no direct manipulation or substitution of genome in the embryo splitting [83, 84]. The splitting of embryos, at the 4-cell stage, into blastomeres can lead to full term, resulting in the birth of many live calves in cattle [85]. The pregnancy rates in bisected early bovine embryos were similar compared with those produced from intact embryos. Under field conditions, the most suitable application is embryo twinning [83]. Furthermore, different age group live monozygotic calves were produced after time-separated thawing and transfer of cryopreserved split embryos [86]. This technique can nearly double the embryos produced from ET and no more than 15 min is required for splitting an embryo [87]. For experiments to be conducted under field conditions, Lopez devised a simple splitting/biopsy procedure [83]. Briefly, 1.5 ml Dulbecco's phosphate buffer saline with no protein was taken in a Petri dish and the placement of embryos undergoing splitting was done in this medium. The embryo was split under the stereomicroscope using 60× magnification and a micromanipulator with a metal microblade. With a single vertical movement of microblade the embryos were divided into two pieces. There was no use of holding pipette while cutting. The biopsy was carried out in the same manner. Compact morulae were bisected in any plane. The positioning of the blastocysts during the cutting procedure was done in such a way as to assure that both trophectoderm and inner cell mass were cut into two equal pieces and the cells that had undergone splitting were separated from the trophectoderm. The attraction of biopsied cells for metal blade was neutralized by adding 1.5 ml of holding medium, containing protein with double concentration, into the dish. The bisected embryos were placed into holding medium in a Petri dish for a few minutes. Transfer of embryos was done non-surgically within 1–3 h using a 0.25 ml straw into the uterus of recipients after synchronization.

Somatic cell nuclear transfer (SCNT)

SCNT is the most common method of cloning in the livestock by which DNA from somatic cell is introduced into enucleated oocyte. Although SCNT is inefficient and expensive, it is successful and has advantages over other breeding schemes [88]. For producing a clone the cost could exceed $10 000 per animal [89]. The number of animals in biomedical research has decreased to 61% over the last 30 years, and cloned animals will further decrease this ratio. The success rate of birth of live offspring from SCNT ranges from 1 to 5% in mammals [90]. Cloned mammals were found to suffer from neonatal development and gestational abnormalities in many studies [91]. Although large offspring syndrome has been found with conventional IVF and ET, the severity and frequency of syndrome was worse in cloning [92].

Transgenesis

Transgenesis is the process of introducing an exogenous gene of interest into the genome of living organism. The first transgenic livestock was produced by injecting the foreign DNA into the pronuclei of zygote in 1985 [93]. The emerging tools for the transgenesis are lentiviral vector, small interferon ribonucleic acid, artificial chromosome as a gene vector and spermatogonial trans-genesis [94]. Lentivirus has played an efficient role as a vector for the insertion of genes into oocytes and zygotes. The birth of the first transgenic cattle was made possible by injecting lentiviral vectors into the perivitelline space of oocytes [95]. Lentivirus has maximized the production of transgenic animals in livestock, because of multiple integration events [96]. In livestock, conditional transgenesis is not feasible because of the long generation intervals [97]. The ability of pluripotent embryonic stem to play a role in organ and even germ cell development following injection into blastocysts is well recognized [98]. It was indicated from the recent data that somatic stem cells have a much broader developmental potential than previously assumed [99]. The gene regulatory process which takes place after transcription and found in fungi, animals and plants is called RNA interference [100]. siRNA-mediated knockdown of the prion protein gene has been accomplished in bovine embryos [101]. The first transposon sufficiently active for use in vertebrates was the Sleeping Beauty (SB) transposon, which was developed in 1997 [102]. The identification of specific pathological phenotypes having a relation with accelerated growth rate in transgenic farm animals has been noted in a previous study. However, the elimination of these problems in subsequent transgenic animals was made possible through modifications in gene constructs. Transgenic farm animals have been shown to mimic human diseases [103]. This technology could be helpful for producing the pharmaceuticals, xenotransplantation and production of transgenic pets.

Stem Cells

The undifferentiated cells having capability to produce unlimited cells of similar nature by differentiation leading to the birth of multicellular organisms are called stem cells. These cells could be preserved from 13 weeks to 3 years in cell culture [104]. Embryonic stem cells and adult (somatic) stem cells are the two classes of stem cells which were developed in animals [105], and include, hematopoietic stem cells, mesenchymal stem cells and tissue-specific stem cells [106]. Mesenchymal stem cells (MSC) have the responsibility for tissue turnover. The proliferation and differentiation of these stem cells lead to their presence in various tissues of body, resulting in tissue repair. The isolation of mesenchymal cells from the adipose tissue can be made possible by treating with collagenase. The most frequent use of collagenase enzyme lies in its collagen cleaving ability [107]. The expression of pluripotency markers such as OCT4, SOX2 and NANOG in bovine mesenchymal stem cells [108] supports the concept of pluripotent potential of mesenchymal stem cells. The establishment of germ lines of embryonic stem cells was achieved in mouse alone amongst all mammals [94]. These cells are found in bone marrow, liver, blood vessels, brain, skeletal muscle and skin. In cattle, bone marrow has been the source for MSC in several studies [109]. The main use of these cells is in cardiovascular disease treatment, brain disease treatment, blood cell treatment and blood deficiency therapy. Stem cell therapy offers potential solutions for a variety of chronic diseases, for which current pharmacologic therapy does not provide effective treatment [110].

Conclusion

All four generations of reproductive biotechnologies have been discussed in relation to their roles in the bovines, keeping in view the status of developed and developing countries such as Pakistan and India in Asia. AI was the first-generation reproductive biotechnology which has a major role in exploiting genetic potential of males. The cost effectiveness made this technology accepted worldwide. Multiple ovulations and ET are a second-generation technology through which genetic potential of females can also be exploited and this technology leads to production of more offspring. High cost and poor superovulatory response of animals has meant that this technique has been less adopted by developing countries. IVEP is the third-generation reproductive biotechnology, but high cost and low pregnancy rates keep this technology limited to developed countries only. Cloning, transgenesis and stem cells are advanced technologies and categorized as fourth generation. These techniques are not possible on practical grounds due to ethics, high cost and low success rates and demand further research.

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