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Animal Reproduction Technologies—Future Perspectives

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Abstract: Reproductive biotechnologies, such as artificial insemination, sperm selection and embryo technologies, offer possibilities for animal producers to increase reproductive efficiency. There have been many significant developments in reproductive biotechnologies over the last few decades, e.g., in sperm handling and preservation, *in vitro* embryo production and preservation, sexing and cloning. This review discusses some of the key changes that have occurred and explores their potential for increasing the reproductive efficiency of domestic animals in the future. As a consequence, they also offer opportunities to facilitate or accelerate genetic selection. If properly used, they may contribute to increase the sustainability of animal production. The role of epigenetics in influencing phenotype is also considered.

Key words: Animal reproduction technologies, livestock, genetic selection, conservation breeding, sperm, embryo production, epigenetics, future perspectives.

1. Introduction

Animal producers are increasingly expected to produce “more for less”, i.e., to have healthy animals that achieve target production levels faster than previously, while consuming fewer resources and producing less waste and fewer greenhouse gases. Producers must meet these high expectations, despite increasingly scarce resources, declining fertility in some species, emerging diseases, environmental toxicology and climate change. Since all animal production starts with reproduction, reproductive biotechnologies can bring about improvements in reproductive efficiency [1]. They can also facilitate the implementation of breeding schemes especially in the context of genomic selection [2]. Importantly, when coupled to genomic selection, reproductive technologies applied to livestock production are key to improving the sustainability of farm animal production [3]. However, the level of development achieved suffers from severe limitations in many species, leading to bottlenecks, especially for the conservation of endangered species and livestock breeds [4]. The purpose of this review is to look at current trends in assisted reproduction technologies (ART) to determine possible future developments.

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2. Animal Reproduction Technologies

The ART considered here include artificial insemination (AI), *in vitro* production of embryos (IVP), embryo transfer (ET), embryo sexing, genotyping and cloning, together with other approaches, such as epigenetics. The advantages and disadvantages of these technologies are presented in Table 1.

2.1 AI

Certain pre-requisites are necessary for AI to be successful, namely, a supply of semen of good quality, reliable methods of oestrus detection in the female or efficient protocols for fixed-time AI, and a means of depositing the semen into a suitable part of the female reproductive tract. Of these factors, only sperm quality will be considered in detail here, since there are several recent reviews providing an update on oestrus

Table 1 Advantages and disadvantages of various ART.

Technique	Advantages	Disadvantages
Artificial insemination	Allows maximal use of superior sires; reduces disease transmission	Only genetic contribution from sire; pathogens can be disseminated in semen
Sperm selection	Selects high quality spermatozoa; removes poor quality spermatozoa, debris and pathogens	Colloids expensive; extra step in semen preparation for insemination doses
Sperm sexing	Maximal use of superior sires; majority of offspring of desired gender	Expensive; lower pregnancy rates than conventional semen; not feasible for all breeds or all males
Embryo transfer	Genetic contributions from both sire and dam; increases genetic progress and availability of commercial products	Specialist skills and equipment required; welfare considerations
Ovum pick-up and *in vitro* production of embryos	Genetic contributions from both sire and dam; increase genetic progress and availability of commercial products; it can be used to deal with genetic variability in selection schemes	Welfare considerations important; specialist skills and equipment required; not possible in all species, e.g., horse
Intracytoplasmic sperm injection	Uses only one spermatozoon; useful for horses (and humans)	Not useful in cattle (disruption of oocyte cytoplasm, lack of activation)
Cloning	Increases commercial availability of animals with high genetic merits	Low success rate; considerable welfare implications; not permitted in some countries; does not favour genetic progress

detection and fixed-time AI, such as Ref. [5]. With regard to sperm quality, relationships have been established between sperm motility, morphology, membrane integrity, chromatin integrity, mitochondrial membrane potential and the likelihood of an inseminated female becoming pregnant and carrying that pregnancy to term. In the past, selective breeding was used in species, such as cattle and pigs, to select males with good sperm quality to be used as breeding sires. There was also a passive selection in bulls for "freezability", in that animals whose ejaculates did not freeze well were not chosen as breeding sires, regardless of other desirable traits. Genomics is now being used extensively to select breeding sires, at least in cattle, which in theory will lead to a wider genetic base and faster rate of improvement. There is interest for genomic selection in other species as well, e.g., dairy breeds of sheep and goats, where it is considered to be more profitable if efficient breeding schemes are already available [2]. However, with less emphasis on selection for sperm quality, there will be greater reliance on selecting good quality spermatozoa. In other species, such as horses and camels, males are still chosen on the basis of their appearance or performance in competition, with the result that advanced semen handling techniques are needed in these species to select good quality spermatozoa and separate them from the rest of the ejaculate.

2.1.1 Sperm Selection

Advanced sperm handling and selection techniques have been reviewed previously [6-8]. Briefly, they are techniques that separate the most "robust" spermatozoa from the rest of the ejaculate. Robustness could include the most motile spermatozoa, or those that are morphologically normal, or with intact acrosomes, or those that survive cryopreservation. Techniques for selection include swim-up, filtration and colloid centrifugation. The most promising of these selection techniques is undoubtedly colloid centrifugation, since it can be used at semen collection stations, particularly the variant called single layer centrifugation (SLC) which uses only one layer of a species-specific colloid instead of several, as in a density gradient. With SLC, it is possible to select highly motile spermatozoa with normal morphology, intact membranes and good chromatin integrity, and to separate them from seminal plasma and the rest of the ejaculate [6-8]. Scaling-up the original technique allows whole ejaculates to be processed, even for

boars where the ejaculate is voluminous [9]. Fertility of the selected sperm samples is improved, compared to unselected samples, e.g., in problem stallions [10] as well as in "normal" ejaculates [11]. Selected spermatozoa also survive cryopreservation better than non-selected spermatozoa, at least in horses [12, 13] and pigs [14]. However, recent studies with bull spermatozoa in a lecithin-containing extender suggest that the selected spermatozoa show increased levels of tyrosine phosphorylation after colloid centrifugation, suggesting that the removal of the seminal plasma leads to the initiation of capacitation, which might not be desirable in sperm samples for artificial insemination [15]. However, it is not yet clear whether this is a feature of bull spermatozoa in general or whether the soy bean lecithin-containing extender plays a role in permitting initiation of capacitation.

The SLC method has also been scaled-down to facilitate processing small volumes (microliters) of semen, e.g., thawed sperm samples for *in vitro* fertilization (IVF). Thus, thawed red deer semen was processed on 1 mL colloid in an Eppendorf tube [16]. However, a comparison of 1 mL of colloid in a 15 mL centrifuge tube and 1 mL of colloid in an Eppendorf tube for thawed bull semen indicated that a higher sperm yield was obtained when the 15 mL centrifuge tube was used [17]. Since the area of the interface between the colloid and semen is greater in the 15 mL tube, presumably there is less competition for the spermatozoa to pass into the colloid than in the Eppendorf tube. This possibility of using less colloid for sperm preparation makes the use of colloids more attractive when preparing sperm samples for IVF.

Apart from sperm quality, improvements in sperm survival during storage are to be expected in the future. There has been interest recently in the influence of season on seminal plasma with its attendant effects on sperm quality. These seasonal changes were observed at least 20 years ago [18], but have received renewed interest recently. It may well be that changes in diet, particularly lipid content, affect the composition of sperm membranes, rendering them more susceptible to damage during freezing. Studies of these effects may lead to identification of additives for diets or for semen extenders to overcome seasonally-induced dietary deficiencies in the composition of sperm membranes, thus improving cryosurvival.

Another suggestion to improve sperm quality is that adding oviductal components, such as heat shock proteins (HSP), may have a beneficial effect on sperm membranes during storage [19]. However, since sperm capacitate under physiological conditions in the oviducts, it is not clear whether adding oviductal components to sperm doses for insemination would necessarily be beneficial, although potential benefits do exist for IVF.

Cryopreservation is the most effective method of extending sperm life. Recent advances in cryopreservation techniques suggest that even spermatozoa from species that were previously considered difficult to freeze can be frozen successfully in the research laboratory. In the near future, it may be possible to develop practical methods for use in the field. New forms of sperm packaging may help to extend sperm life. Recently, the possibility of encapsulating spermatozoa was suggested as a means of providing slow release of spermatozoa into the female reproductive tract. Although preliminary fertility data in cattle and pigs look promising [20], it remains to be seen whether this form of packaging will be useful for the AI industry.

2.1.2 Sperm Sexing

Pre-conception gender selection has been a goal for many years. Many animal production systems make use of only one gender, e.g., dairy cows for milk production and hens for egg-laying, but even in pig production units, the ability to select for female offspring would obviate the need for surgical castration of male piglets. Some species of fish stop growing on attaining sexual maturity, which means

that females (which mature later than males) tend to achieve larger body weights and therefore have a higher carcass value than males. In many instances, the birth of offspring that are not of the desired gender leads to production inefficiencies and wastage, and hence pre-conception gender selection is likely to be one of the important areas of focus in the coming decades. Although sperm sexing would not allow gender selection of poultry (where the female is the heterogametic sex), it would be effective for mammals and has therefore received a lot of attention in the last few decades. The only method of sexing spermatozoa, which has been shown to work reliably so far, is the flow sorting of spermatozoa [21, 22] stained with the vital dye, H33342 [23]. However, the process of sorting sufficient sperm numbers for an insemination dose takes a long time, since the stained spermatozoa must pass individually through a laser beam for detection of their DNA content. Although flow cytometric sexing of spermatozoa is 70%-90% reliable, the method is slow and expensive, and sperm fertility may be reduced. Preliminary data on sexing of boar spermatozoa using antibodies to the recently discovered sperm-surface proteins [24] suggest that an alternative route to achieve sperm sexing may be available in the future.

2.2 Embryo Technologies

Embryos can be obtained from IVP, IVF or intracytoplasmic sperm injection (ICSI). IVP typically involves the *in vitro* maturation of immature oocytes obtained from slaughterhouse material, followed by IVF and culture of the resulting zygotes before transferring to recipients or freezing for later transfer. Although these techniques have been available for several decades, their use in animal production systems is still limited by the cost of techniques, and is almost exclusive in cattle. In Europe (France, Germany, the Netherlands), they are now extensively used in dairy cow selection schemes. In South America, mainly Brazil, there has been a huge commercial development for beef production over the last decade, although there has recently been a shift in predominance towards dairy breeds [5]. Although the production of large offspring and associated syndrome of the neonate is now avoided by use of media without fetal calf serum (FCS), there are still some problems to be resolved and the efficiency of the system requires optimization to reduce the cost. Oocyte quality varies considerably depending on a huge number of factors, which influences the subsequent outcome of ET. Many epigenetic factors known to affect oocyte function and early embryonic development (see section on epigenetics) are also thought to affect the health of the resulting offspring. It is possible that IVP embryos are more at risk of adverse effects because of lack of control mechanisms that would be present *in vivo*.

Despite the considerable interest in using IVP in the production of transgenic pigs during the 90s, the technique of IVP for pig embryos is currently not as well developed as for cattle embryos. The problem of polyspermy is still a limiting factor in this species and *in vitro* culture is not considered to be optimal yet [25]. On-going research may overcome the polyspermy problem in the future, thus enabling more use to be made of IVP in pigs.

Ovum pick-up (OPU) for subsequent IVF and ET is increasing in Europe and North America, and has the potential for obtaining many more offspring from cows of known genetic value than they could produce physiologically. It is rapidly outpacing conventional superovulation for ET, having increased more than ten-fold since the turn of century [26]. The technique uses ultrasound-guided follicular aspiration to recover oocytes from pre-ovulatory follicles *in situ*, and can even be used on pregnant cows. Twice weekly OPU can be practiced without any side effects that could compromise subsequent embryo development. In some circumstances, OPU can be combined with

superovulation to increase the number of oocytes recovered. The remarkable increase in its use in Brazil is attributed to it being more effective and cost efficient than superovulation in *Bos indicus* cattle.

In Europe, some companies work mainly with *in vivo* produced embryos, whereas others have integrated IVP in their systems. However, there is a strong need for companies to develop and optimize the efficiency of embryo technologies in order to be competitive. Most particularly, the variation in pregnancy rates obtained after the transfer of IVP, biopsied and cryopreserved embryos (the most interesting strategy from a genetic point of view) still represents a major bottleneck in the use of these technologies.

OPU has been used in other ruminants, such as buffalo, although the ovaries are small compared to cows and contain fewer follicles. The follicular population may be influenced by season [27]. In contrast, superovulation with subsequent ET has no great impact in this species, because of the limited number of embryos recovered and their low survival after cryopreservation [28]. However, OPU is still the only reliable method for obtaining oocytes for equine IVF, since IVP of horse oocytes tends to result in hardening of the zona, thus preventing sperm penetration. Normally only one pre-ovulatory follicle is present in each cycle during the breeding season, thus making the yield from this technique less effective than in cattle. However, the yield of useable oocytes can be increased by aspiration from all follicles greater than 1 mm in diameter [26, 29]. Researchers are still continuing their attempts to develop equine IVF, although, to date, only a few foals have been born using the technique, despite many attempts. Moreover, IVP allows the production of some hybrids, e.g., camas (camel/llama hybrids), although such applications are research-based rather than being of practical use for animal production at present. Similarly, the techniques may be used for species conservation. In contrast to equine ICSI, bovine ICSI has not progressed into clinical practice. The lack of success in producing offspring is generally attributed to inadequate oocyte activation [30] or to cytoskeletal damage, e.g., from the large diameter pipette needed to accommodate the bull spermatozoon [31]. Although advances in oocyte activation may allow more embryos to develop after ICSI, the ease and availability of bovine IVF provides little incentive to develop the more laborious technique of ICSI in this species.

Use of sperm sexing in association with IVF-IVP may also avoid some of the present limitations in selection schemes, due to the high number of spermatozoa that must be discarded and the large individual variation associated with the sexing process by flow cytometry [32]. Sexing of embryos enables only those of the desired gender to be transferred. However, the procedure is time-consuming and requires considerable skill to avoid harming the embryo. The procedure involves removing some cells from the embryo; the DNA from the biopsy is then processed by polymerase chain reaction (PCR) for identification of X- or Y-chromosome specific sequences, whilst the embryo is frozen or vitrified. Once the gender of the embryo has been determined, the embryo can be transferred to a recipient or destroyed. New techniques have been used with horse embryos that permit sexing to be done with a reasonable degree of accuracy in 6-10 h, whilst the embryo is maintained in cultured. Thus, the embryo can be transferred on the same day as the biopsy [33], which is a significant development in this species. Presumably the technique could also be modified for use in other domestic species.

2.3 Cloning

Somatic cell nuclear transfer (SCNT) has been carried out in cattle and other livestock, but the procedure remains technically demanding and expensive. A donor cell is injected into or fused with a

mature, good quality, enucleated oocyte, followed by activation and culture of the product. However, there are high rates of pregnancy loss and also abnormal placental development and pathologies during the neonatal period, which is a major limiting factor in the adoption of this technique.

This technique may allow a wider diffusion of animals of interest, and some results have been obtained in the preservation of endangered species. However, the efficiency of the process is not high enough to reconstitute viable populations. The technique also has considerable limitations with regard to genetic progress, since genetic variability is an important component even in the context of genomic selection [34]. It is possible that future developments will enable the technique to achieve the levels of efficiency and reproducibility necessary to produce live offspring consistently. Considering the need to maximize genetic variability, cloning is unlikely, at least at present, to represent a useful tool in selection schemes organized by breeding associations and companies due to limitations in reproductive efficiency. However, individual farmers with access to genomic selection may be interested in the duplication of their best cows through cloning for commercial purposes in countries allowing the use of this process.

Much attention has been focused on cloning in pigs but with relatively little success in terms of live piglets. Currently, only 1%-5% of SCNT embryos develop to become piglets [25]. However, increased success has been reported using zona-free reconstructed embryos, which may eventually lead to improvements and eventual optimization of the process.

3. Use of Embryo-Based Biotechnologies in Genomic Selection

In an attempt to improve numerous traits by genomic selection, knowledge of the relationships between genome information and phenotypic criteria is of crucial importance. Initially, microarrays were used to characterize the relationships between genotype and phenotype [35, 36]. More recently, high-throughput technologies for DNA and RNA sequencing analysis have been used to study relationships between genotype, phenotype and gene expression. With these objectives, phenotyping (animal models, precise criteria and methods) becomes the main bottleneck to achieve this goal. As a consequence, research is needed to phenotype new critical traits and to improve the precision of the phenotypes for existing traits, such as fertility and reproductive traits [37, 38]. For this purpose, proteomics, lipidomics and metabolomics may be particularly appropriate to find new markers for fertility [38] or for predicting embryo survival potential and the ability of recipient to sustain pregnancy to term [39, 40].

One of the most important features of the new selection procedures will be to increase the number of candidates submitted for genomic selection, to maximize the chances of producing interesting individuals that will be evaluated positively for a large number of traits. As mentioned before, this will allow an increase in the selection pressure for those traits. In addition, it will be possible to use bulls for AI at a younger age, thereby lowering the generation interval. Finally, the use of groups of bulls with a favourable genomic index will improve the precision of indices, when compared to the use of a very limited number of older sires, as was the case in the past. This may be favourable to genetic variability if adequate and wise breeding schemes are implemented; otherwise shortening the generation interval may also lead to an increased inbreeding rate.

The method of producing the large numbers of animals to be genotyped may become a rate-limiting factor. Thus AI alone may be inadequate to generate sufficient animals in a given period of time, and the efficiency of multiple ovulation and embryo transfer

(MOET) and OPU-IVP will become more important. With these "intensive" embryo-based reproductive techniques, it is relatively easy to increase the number of candidates by increasing the number of flushes in MOET schemes. However, compared to MOET, two to three times the number of embryos can be produced in a given period of time by use of repeated OPU-IVF sessions [41, 42]. This method also presents advantages in preserving genetic variability. Much research has been done to improve *in vitro* culture systems. This allows most teams working with IVF to obtain overall development rates up to the blastocyst stage of between 30% and 40%.

The effect of a previous superovulation on fertilization and subsequent embryonic development is still controversial [43]. It has been shown very clearly from most studies that there is a significant decrease in embryo production when oocytes are matured *in vitro* in standard medium compared to *in vivo* conditions [43, 44]. This emphasizes the roles of the final steps of oocyte growth and maturation on subsequent embryo development, which have also been illustrated by epidemiological studies showing relationships between some factors influencing these steps and embryonic mortality [45]. It is likely that much progress can be achieved in embryo production by optimizing the conditions under which the oocytes are growing within follicles in donor females. Handling at the time of collection, and thereafter, as well as during *in vitro* maturation, requires optimization since dramatic metabolic changes occur very quickly after oocyte recovery [45].

Despite these limitations, the work that has been done in the past 15 years to improve oocyte collection and *in vitro* embryo production systems has enabled the most advanced breeding companies to produce more embryos in their genetic schemes [41, 46]. However, there are also disadvantages, such as miss-management of the use of these techniques, that may lead to a significant increase in inbreeding, especially if bull dams are over-exploited. Moreover, due to new requirements in relation to the implementation of genomic selection (increased number of candidates), additional limitations exist for producing the very large number of calves that would be genotyped after birth. Effectively, one of the main bottlenecks experienced by breeding organizations working with dairy cattle in Europe is the limited availability of recipients. This is reinforced by the fact that not only are lower pregnancy rates achieved when using cows as recipients instead of heifers, but also the efficiency of ET is much lower if heifers are used as donors [42]. In addition, high costs will be induced by the transfer of a very large number of embryos into recipients that must be maintained until birth of the progeny, and the economic potential of the non-selected calves will be low. When producing these candidate animals on farms, the amount of field work in relation to ET and *in vitro* production will be even greater than at present and will generate high logistical costs.

Limitations in embryo production are also encountered due to use of young animals to reduce the generation interval. Genomics allow early identification of candidates for the selection scheme, but the challenge is now to produce enough good quality gametes and embryos from young animals. Thus, a very important research area is how to obtain gametes from pre-pubertal animals and/or to hasten puberty. However, it is also important to balance the desire for early puberty against the long term health and productivity of the animal [47]. Thus females should not be bred at their first ovulation, but should be given time to enable regular ovarian cycles to become established and for the uterus to become capable of supporting a pregnancy. In parallel, it is critical to develop tools to predict the donor's superovulation responses to avoid the inefficient treatment of poor responders, and thus to decrease the costs of selection schemes.

Table 2 Pregnancy rates (day 90 post transfer) following transfer of fresh or frozen biopsied embryos.

Location	Pregnancy rates	
	Transfer of frozen biopsied embryos	Transfer of fresh biopsied embryos
Farm	61/116 (52.3%)	109/171 (63.7%)
Station 1	46/83 (55.4%)	
Station 2	28/54 (52.0%)	
Total	135/253 (53.0%)	109/171 (63.7%)

Results from Farm and Station 1 were obtained after embryo sexing. Results from Station 2 were obtained after biopsy and typing for 45 microsatellites (detection rate 98%), modified from Ref. [30]. Farm refers to field conditions, whereas Station 1 and 2 refer to research stations where the conditions may be different to those found in the field.

Finally, this process may increase the contractual cost for individual farmers especially due to the potential existence of very interesting candidates identified by genomics. For these reasons, genotyping the embryos and selecting them before transfer appear to be an attractive scenario to maximize the chances of finding interesting individuals for multiple traits, while transferring a "reasonable" number of embryos.

4. Embryo Typing

The interest of embryo typing for breeding companies was discussed long before the emergence of the new techniques for genomic selection that currently includes thousands of markers [41]. Typing and selection early in life was expected to be a solution to shorten the generation interval, to limit the costs of producing high numbers of calves and progeny testing to achieve multi-character selection. Today, the potential advantages of combining intensive embryo production and genotyping are even higher. Results reported initially in the literature for ruminants were based on typing for a limited number of markers [48]. Field studies with *in vivo* produced, biopsied embryos (either fresh or frozen) have shown that pregnancy rates following transfer on-farm were compatible with field use [34] (Table 2).

Initially, typing was envisaged from a large number of cells obtained from reconstituted embryos following cloning of blastomeres. Since then, preliminary studies from limited numbers of biopsies and typing have shown that the use of pre-amplified DNA is possible [34] and also compatible with typing from high density marker chips. Thus, it may be useful to perform economic and genetic simulations to evaluate the costs and advantages for the genetic schemes of such procedures based on embryo typing.

5. Epigenetics: Perspectives for Its Use

Epigenetic changes are stable alterations of DNA-associated molecules that do not involve changes of the actual genes. The expression of genes can be altered due to modifications of active regulatory sequences inducing alterations of the cellular phenotype, which can in turn lead to modifications of animal phenotype. Epigenetic alterations involve changes in DNA methylation (including global hypomethylation and more locus specific hypermethylation) and methylation or acetylation of histones [49, 50]. They can occur in all cells in the body, but if they occur in oocytes or spermatozoa, they may be passed on to the next generation. The epigenome is especially vulnerable during embryogenesis, fetal and neonatal life and at puberty [51, 52]. Effectively, the epigenome undergoes extensive reprogramming when the gametes fuse at fertilization (during the final stages of meiosis and around fertilization due to chromosomal decondensation and intense remodelling) and during the preimplantation period. These epigenetic changes are necessary for normal embryonic development and survival [53]. The modification of epigenetic marks

that occurs during preimplantation development are correlated with the activation of the embryonic genome [54, 55].

After fertilization, different patterns of methylation exist for some epigenetic markers, followed by more or less early demethylation, which allows transcriptional activity of the embryonic genome [56]. Later, at the morula stage, DNA methylation at specific loci influences differential gene expression patterns of the cells at the periphery of the embryo [57], whereas cells at the center of the embryo do not receive the same environmental influence and instead conserve their totipotency. Reproductive technologies are used during stages of fertilization and early embryo development, when a potential window of vulnerability exists. Experimental models have been used to determine the role of epigenetic effects on embryonic development, and a large number of studies demonstrate the impact of ART on gene expression in the mouse. In the mouse and cow, epigenetic modifications induced by ART were associated with impaired early embryonic survival, but also with deleterious effects on further post-implantation, fetal, placental and postnatal development [58-60]. In these species, as well as in humans, some alterations may be due to changes related to the epigenetic regulation of endometrial function [46]. Thus, epigenetic effects may influence reproductive efficiency through alterations in the viability of embryos, foetus and neonates, and the control of endometrial gene expression which may modify implantation. They may also influence health, especially the occurrence of cancer through regulation of proto oncogenes and suppression of tumour suppressor genes, and phenotypic performance for a variety of functions or traits. Some epigenetic modifications can even be transmitted to subsequent generations, leading to remnant alterations of the phenotype within families.

Numerous factors can induce epigenetic effects: evidence exists for the role of nutrition, either over-nutrition increasing the rates of diabetes and obesity, or under-nutrition. Nutritional challenges to established germ cells can determine the chromatin structure, leading to metabolic responses throughout life in an individual. Moreover, endocrine disruptors, which can induce durable changes in receptor sensitivity to steroids (androgens and oestrogens), may be involved in cancer occurrence, and various pollutants can also induce such effects. Thus epigenetic regulation of gene function is a key factor in understanding the interactions between the environment and genome function [61]. This has clear implications in selection, especially nowadays when using genomics based on DNA sequence characteristics to predict future performance instead of observed phenotypic performances of offspring through progeny testing, which were integrating potential epigenetic effects in the evaluation.

Considering all this information, a better knowledge of epigenetic effects when using ART will also help to define culture conditions for oocytes and embryos that will not impair the subsequent development of embryo, foetus or neonate, or the health of the offspring. More generally, this knowledge may help to define preventive measures, which may be favourable to fertility and health for a variety of species including man.

6. Societal Pressures

Societal concerns must also be taken into consideration. Consumers are concerned about the long term consequences and safety of different techniques. There can be an unfavourable perception of the use of embryo technologies, e.g., possibly being associated with a reduction of genetic variability, use of hormones in the protocols and induction of undesired epigenetic effects. On the other hand, their use can help to answer some of the major issues raised by society. Use of ART combined with genomics

allows selection for new traits, such as disease resistance, feed conversion efficiency, reduction of methane emission and quality of product, all of which help to meet societal demands. Therefore, communication with stakeholders is vital to transmit the message that these technological advances are helpful when used correctly, and of course, there should be adequate training of personnel involved in using these techniques.

7. Conclusions

There are many potential uses for reproductive biotechnologies in livestock production, and their use is likely to increase. Sperm selection techniques are likely to become more widely used to increase reproductive efficiency in inseminated animals. The tremendous expansion of ET in South America may indicate the future direction of these technologies in other areas of the world. Genomic selection will continue to be important in the immediate future, but more knowledge about the effects of epigenomics is needed to understand the potential effects of the environment on specific genotypes. Furthermore, possible epigenetic effects associated with their use should be more intensively studied to increase knowledge about factors conditioning the health of offspring over generations and improve public acceptance.

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