Use of Advanced Reproductive Technologies and Inclusion of these Records in Genetic Evaluation—R. Mark Thallman and Alexandria Snider

Introduction

Most of the performance records of the millions of seedstock cattle produced by embryo transfer over the past 50 years have been excluded from national cattle evaluation (NCE). A topic entitled "Embryo Transfer (ET): Data Collection and Utilization" was recently added to the BIF Guidelines to provide recommendations on the utilization in genetic evaluation of records of cattle produced by embryo transfer.

Here, we provide background information on the use of embryo transfer in cattle breeding, various forms of ET and terminology used to describe them, and their relative advantages and disadvantages. Based on this background, we explain the rationale behind the recommendations in the current BIF Guidelines. We also discuss a few topics related to ET, but not addressed in the current Guidelines and propose actions that could allow records of additional ET cattle to be included in future genetic evaluations.

Embryo Transfer

Embryo Transfer refers collectively to a set of reproductive technologies to increase the reproductive rate of cows. Embryo transfer was commercialized in the 1970s and has contributed substantially to the genetic improvement of beef cattle since the 1980s. Selection of donors and service sires for ET should follow the principles of the BIF Guidelines.

Roughly 100,000 beef seedstock calves are produced annually in the U.S. through ET. Although this represents a minority of beef seedstock calves, they are disproportionately influential because a large proportion of AI sires, as well as other sires used in seedstock herds and donor females, are themselves produced through ET. Therefore, it is important that these influential parents be selected from the pool of candidates as accurately as possible. Furthermore, ET calves are more likely to be measured for expensive traits such as individual feed intake.

Embryos are placed (usually at day 7 of development) into cows (the recipients) that carry the fetuses and raise the calves. The recipient cows are typically not related to the embryos. To be a viable recipient, a cow must be "in synchrony" with the embryo, which usually means she was in estrus 7 days prior to transfer. More calves can be obtained from cows of superior breeding value by these techniques. Embryo transfer (ET) can improve selection response through increasing selection intensity, reducing generation interval, and increasing the accuracy of dams.

Most variations in ET are related to the source and/or processing of the embryos, which are described below.

Multiple Ovulation Embryo Transfer (MOET)

Historically, most embryos for ET have been produced through MOET. The embryos are typically flushed from the uterus of their genetic dam (the donor) 7 days after AI; the donor is usually superovulated by injection with follicle stimulating hormone (FSH) prior to AI so that multiple embryos are collected. The number of transferable embryos per collection is highly variable[3] and influenced by many factors but averages about 7. The donor cow must be open and cycling to produce embryos through MOET. Donor cows can be collected multiple times between pregnancies, usually about 45-60 days between collections. Pubertal heifers can be collected; they usually produce fewer transferable embryos than cows.

MOET is sometimes referred to in industry as "conventional ET" or "in vivo production" of embryos. The process of collecting embryos in MOET is referred to as "flushing" because the embryos are literally flushed from the donor's uterus in a special collection fluid. Consequently, the MOET technique is sometimes referred to as "flushing" to distinguish it from in vitro production (see below).

In Vitro Fertilization (IVF)

Alternatively, embryos can be produced by IVF, which makes it possible for donors to be collected for most of the year while keeping them in an annual calving season. It can also allow production of multiple progeny per straw of semen[3].

In IVF, unfertilized oocytes are collected from the donor cow's ovaries by transvaginal, ultrasound-guided needle aspiration of multiple follicles per ovary. This process is referred to as ovum pickup (OPU). Because most of these follicles would not have ovulated naturally, they must undergo in vitro maturation (IVM), then in vitro fertilization with bull sperm, followed by in vitro culture (IVC) in a laboratory until being ready for transfer into recipient cows at the blastocyst stage (about day 7).

Technically, the entire process is referred to as in vitro production (IVP) and IVF refers specifically to the fertilization aspect of the process. In industry, the entire IVP process is more commonly referred to as "IVF" and that use of the term is adopted in the remainder of this document. Methods of collection are sometimes distinguished as in vivo (MOET) versus in vitro (IVF).

In 2019, 96,887 IVF beef embryos were transferred in the U.S., up from 41,993 in 2015[4]. This increase is partially at the expense of MOET embryos. In 2019, 109,218 MOET beef embryos were transferred in the U.S., down from a high of 156,506 in 2015[4].

The increase in use of IVF is likely due to the opportunity to collect donors without disrupting their normal calving season as well as the opportunity for greater (and perhaps more uniform) annual embryo production per donor. In IVF, the donor need not be in estrus and even pregnant cows can be collected via OPU. Donors are typically collected every 2 weeks and produce approximately 8[4] transferable embryos per collection.

Donors for IVF can be treated with FSH prior to OPU, or not. Using FSH increased average viable embryos per OPU from 5.0 to 8.4 but may have long-term effects on donor cows and unknown effects on resulting progeny. In 2019, 170,924

viable IVF embryos were produced by companies that predominantly use FSH and 23,146 were produced by companies that predominantly don't use FSH[4].

Juvenile In Vitro Embryo Transfer (JIVET)

Prepubertal heifers can be collected (with decreasing success at younger ages); this form of IVF is known as juvenile in vitro embryo transfer (JIVET). In principle, JIVET could decrease generation interval on the female side of the pedigree to one year; this could make it a very useful tool for cattle breeders. The IVM step in JIVET is more technically demanding than in IVF from pubertal donors.

Calves produced through IVF may be subject to Large Offspring Syndrome (see below). It seems plausible that JIVET may accentuate large offspring syndrome relative to pubertal IVF, but this appears to have not yet been evaluated. Combined with genomic selection, the reduced generation interval made possible by JIVET could substantially increase response to selection. It could also cut approximately in half the minimum time required to introgress a rare allele into a more useful genetic background.

Nuclear Transfer (NT)

Nuclear transfer, commonly referred to as "cloning", can be used to produce groups of genetically identical individuals. Somatic cell nuclear transfer can be used to produce animals genetically identical to existing animals, including those that have been neutered, become infertile or unhealthy, or that have died (provided an appropriate tissue sample is collected and handled properly soon enough postmortem).

In nuclear transfer, embryos are produced by fusing the nucleus of a donor cell with an oocyte from which the nucleus has been removed, resulting in a cell very roughly equivalent developmentally to a fertilized one-cell embryo. These embryos are then cultured in vitro (similar to IVF) to the blastocyst stage prior to being transferred or frozen. Donor cells can be relatively undifferentiated cells in embryos or somatic cells of live cattle of any age or sex (including steers). The latter process, referred to as somatic cell nuclear transfer (SCNT) was used to produce "Dolly" the famous sheep and a few years later 8 cloned calves. The somatic cells are often cultured from skin cells from ear notches. The oocytes to which donor cells are fused are typically collected from ovaries recovered in large numbers from cow harvesting facilities; little is typically known about the oocyte donors other than whether they are heifers or cows and whether they are beef or Holstein. Cattle produced by NT are (for practical purposes) genetically identical to the donor; in principle, large numbers of genetically identical progeny could be produced. A variation on SCNT referred to as "handmade cloning" is reported to be easier to perform and more efficient than SCNT. Because of currently high cost per pregnancy, use of NT is generally limited to reproducing highly valuable individuals that have died or become infertile; it has been used to clone extremely desirable (e.g. Prime YG1) carcasses identified on the grading rail [10]. Perhaps the most common current use of SCNT is to facilitate gene editing. Calves produced through NT may be subject to Large Offspring Syndrome and Abnormal Clone Syndrome (see below). Van der Berg et al. (2019) is an excellent review of technical, safety, ethical, and regulatory aspects of SCNT as well as a review of companies commercially engaged in SCNT.

Freezing

Embryos can be cryopreserved (frozen in liquid nitrogen) to be transferred at a more convenient time. This allows donors to be collected throughout the year, potentially generating a large number of candidate embryos, while the recipient cows calve during the optimal calving season. Having an inventory of frozen embryos also ensures that a high-quality embryo is available to transfer to each recipient cow and that transfers are timed optimally relative to estrus of the recipients.

Transfers of embryos that have not been frozen are referred to as "fresh" transfers. Frozen embryos may be referred to as "vitrified". In 2019, 70% of beef MOET transfers and 67% of beef IVF transfers were of frozen embryos[4]. Pregnancy rates are approximately 60-70% for MOET fresh and 50-60% for MOET frozen transfers and about 10% lower for IVF transfers. Freezing is used more widely in beef than in dairy cattle[4] because it facilitates seasonal calving. Freezing also facilitates more precise synchrony of the embryo with the recipient.

Calves resulting from frozen embryos had greater gestation length and were heavier at birth than those from fresh transfers in some circumstances, but not in others. Differences in phenotype between calves resulting from fresh or frozen transfers are not widely recognized but may exist. In humans, children born from frozen IVF embryos had greater birth weight than those born from fresh IVF embryos.

Sex Determination and Genotyping

The opportunity to preselect embryos offers the potential to greatly increase selection intensity with relatively little increase in cost. The current impediment to widespread adoption is genotyping cost per calf if intensive selection is applied to embryos.

One or a few cells can be removed from an embryo prior to transfer or freezing to be used for DNA analysis. Applications include sex determination, genotyping, and low-pass sequencing. Although this could be feasible without freezing the embryos, it is more practical in conjunction with freezing.

Large Offspring Syndrome

Calves produced through NT or IVF may be subject to Large Offspring Syndrome (LOS), resulting in increased gestation length, birth weight, dystocia, abortion, higher postnatal mortality rate and a wide variety of congenital abnormalities[31]. Large offspring syndrome in cattle is sometimes referred to as "large calf syndrome".

Large Offspring Syndrome is primarily associated with gestation length, birth weight, dystocia, and neonatal characteristics, but although effects on phenotypes measured later in life may be relatively smaller, they should not be assumed negligible. Large offspring syndrome is the result of major disruptions in embryonic and fetal development and its effects should be expected to persist throughout life.

Birth weight of IVF calves can vary depending on the type of culture media used[34] and other conditions (e.g., oxygen tension), and the in vitro maturation (IVM) conditions and it's duration.

Much has been learned about potential causes of LOS, so the severity and/or frequency of it may currently be considerably less than in the past. Hopefully, IVC techniques can be modified sufficiently that LOS is no longer an issue.

Abnormal Clone Syndrome

Extremely high birth weights, prolonged gestation, failure to initiate parturition, and many other abnormalities were first recognized in calves cloned from embryonic cells in the 1980s. It later came to be known as Large Calf Syndrome, or more commonly, LOS to refer also to sheep and other species. For a long time, it was thought that SCNT calves suffered a more severe or frequently occurring[25] form of LOS than IVF calves. More recently, it has been recognized that, in addition to LOS, there is a separate set of abnormalities, primarily fetal and placental abnormalities, that can occur in calves produced by NT that do not typically occur with IVF[25]. This is referred to here as abnormal clone syndrome (ACS), but there does not seem to be a consensus in the literature on how to refer to it.

The list of abnormalities in NT, but not IVF calves is not well defined but seems to include failure to initiate parturition, enlarged umbilical cord that required clamping and sometimes urachus surgery, respiratory problems[46], lethargy[46], contracted flexor tendons[46], and other congenital abnormalities.

These additional abnormalities are thought to be due to incomplete reprogramming of the DNA in the fusion of the donor and enucleated oocyte cells to the epigenetic state of a fertilized one-cell embryo. This is a major challenge in NT that is not required by IVF. The characteristics of LOS common to IVF and NT are assumed due to in vitro oocyte maturation and in vitro embryo culture, which encompasses most of the duration of development in both processes.

Calves produced by NT vary greatly in the extent to which they are affected by LOS and ACS, with some appearing completely normal while the most severely affected may be twice the weight of a normal calf.

Progeny of SCNT cattle generally seem to be relatively unaffected by LOS and ACS[36].

It seems reasonable to assume that abnormalities observed in IVF calves also occur in NT, but it does not seem reasonable to assume that abnormalities observed in NT calves also occur in IVF calves, even if the assumption is that the frequency or severity is lower in IVF.

The prospects for modifying IVC techniques such that LOS is eliminated from IVF are far better than the prospects that modifying SCNT techniques will eliminate ACS. The ongoing process of improving IVM and IVC techniques is a matter of making the culture environment more like the natural environment and much progress has already been made. In contrast, the epigenetic reprogramming required for NT is much more challenging because it is a process that does not occur in nature. The more we know about SCNT, the more astonishing it is that it works at all.

Modelling Records of ET Animals in Genetic Evaluation

Throughout the history of genetic evaluation of beef cattle, many cattle have been selected for use as sires and donors in seedstock herds based on EPDs that did not reflect their individual performance. The aim of this Guideline is to reduce that effect to the extent possible.

Seedstock animals resulting from ET are potentially influential and reflect additional investment to achieve genetic progress. Therefore, maximizing the accuracy of genetic predictions early in the animals' lives by using the animals' own observations has increased importance. But, for maternally influenced traits such as weaning weight, the genetic evaluation model must be modified slightly to account separately for the donor's contribution to the calves' genetics and the recipient cows' contributions to maternal environment.

Methods for modelling the effects of recipient dams are in the literature and can be easily incorporated in genetic evaluations. Specifically, both the maternal additive genetic effect and the permanent maternal environment effect should be associated with the recipient dam instead of the donor dam.

Recipient Effects in Genetic Evaluation

Effects on the phenotype due to the dam of the animal are present in traits measured up to weaning, but generally not seen on phenotypes measured post-weaning. For animals produced using ET, these maternal influences are primarily due to the recipient dam, rather than the embryo donor dam. Ideally, pedigree information on the recipient would be included but it is not always available, as recipients are often commercial females. Both age of the recipient dam and its breed composition affect maternally influenced traits - i.e. birth weight, calving ease, and weaning weight. Therefore, if recipients of mixed breed composition or parity group (1st, 2nd, or later) produce calves contemporaneously, the differences in breed and/or parity among recipients should also be reported to the breed association and accounted for in genetic evaluation models.

Suitability of MOET Records for Genetic Evaluation

It has been reported[43] that calves produced by MOET are substantially heavier at birth than non-ET calves due to the time outside the cow between collection and transfer, although the data structure was far from ideal for estimating such effect due to separate management of MOET and non-ET progeny (both reports are from different analyses of different subsets of the same population). Based on knowledge gained through studying LOS, it seems plausible that the transfer medium, exposure to atmospheric oxygen, etc. for the few hours between collection and transfer (or freezing) could alter embryonic and/or placental development, resulting in increased birth weight. Consequently, the prudent assumption (until disproven) is that mean differences exist between MOET and non-ET phenotypes for all traits. There likely is ample data (limited by the co-occurrence of both types in the same contemporary group and code) to evaluate this assumption in existing field data; this would be a very useful exercise. Until this is done, the following recommendations are based on the more cautious approach.

Nonetheless, the data structure in [51] was well-suited for estimation of heritability in subsets of the data. Heritability of birth weight of non-ET calves, and MOET calves with Holstein, beef crossbred, or unknown breed recipients was 41.4±4.3, 28.4±3.1, 32.4±3.8, and 32.5±3.4%, respectively[51]. The MOET calves resulted from transfers of mixtures of fresh and frozen, sexed and un-sexed embryos and probably countless other variations in MOET processes, none of which were available for the analysis. This missing information probably contributed to the lower heritability of MOET calves compared with non-ET calves. Thus, birth weight records from calves produced by MOET are suitable for use in genetic evaluation even with little or no information on the recipient breed and age (excluding heifers) or the variations of MOET techniques performed. In such cases, it would be preferable to fit additional residual and/or permanent environment variance to the model for such records. Nonetheless, it is far preferable to have as much information as possible on the recipient cows, and where feasible, to use registered recipients that have several previous recorded calves. Furthermore, it would be useful to record whether MOET calves were produced from fresh or frozen transfers, were biopsied for sex determination and/or genotyping, and whether any other substantial variations in ET technique were performed.

Suitability of IVF Records for Genetic Evaluation

The commercial use of IVF by seedstock producers is increasing rapidly for the reasons discussed above. Unfortunately, innumerable reports in the literature suggest that LOS makes phenotypes of IVF unsuitable for inclusion in genetic evaluation. Much has been learned about causes of LOS and techniques have been modified to reduce its impact. Anecdotal information suggests the prevalence and/or severity of LOS has decreased substantially since the early days of IVF. Nonetheless, sufficient evidence to warrant inclusion of IVF phenotypes in genetic evaluation is not currently evident.

Because of the importance of IVF to genetic improvement, efforts should be made to utilize those phenotypes as soon as it becomes feasible. A first step could be to estimate heritabilities and genetic correlations to the same traits in non-ET calves using IVF and MOET field data as it currently exists. At least one breed association currently records whether ET calves were produced by MOET or IVF. For associations that do not already record it, this information should be available to the breeder, at least if the breeder owned the donor at the time of collection, so it could be obtained retrospectively, if there was sufficient motivation to do so. The analysis required to directly quantify the effects of both MOET and IVF on phenotypes of traits in NCE from field data would not be trivial but appears feasible.

It should be expected that heritabilities of ET records would improve if more details on the techniques used to produce each calf were available for inclusion in the analysis but this would require additional transfer of information from ET provider to breeder to breed association to genetic evaluation provider. Accomplishing this transfer of information is not trivial, but it could greatly accelerate the incorporation of IVF phenotypes in evaluations and could also provide feedback to ET providers on which techniques are most effective in reducing or eliminating LOS.

The DNA of calves affected by LOS tend to have some characteristic epigenetic marks. It is possible that these or other biomarkers could predict the degree to which individuals are affected by LOS. If so, phenotypes of the most severely afflicted calves could be eliminated and those of many calves could potentially be used in genetic evaluation, perhaps adjusting for degree of affliction. This would require collection of tissues samples for specialized DNA analysis that is different from routine genotyping, but it might be a feasible way to utilize records of IVF calves.

Suitability of NT Records for Genetic Evaluation

Current evidence suggests that the effects of ACS and LOS are too frequently severe to consider including NT records in genetic evaluation in the near future. Hopefully, that situation will eventually change.

Genetically Identical Animals

Although NT records are not used in most genetic evaluations, groups of genetically identical animals (often resulting from NT) do appear in the pedigrees of genetic evaluations. Breed associations differ in how they handle genetic identicals in the pedigree.

Some treat them as different individuals with the same parents, i.e., as full sibs. This approach results in identicals that have produced progeny having different EPDS. If we don't believe that clones are identical, this approach allows us to compare their EPDS. However, quantifying the degree to which EPDs of a pair of identicals fit as full sibs differ from each other due to chance, conditional on their accuracies is not a trivial task, and even if we completed it, far too few such pairs exist in current field data to reach a valid conclusion from them.

To understand some disadvantages of this approach, consider the following example: a popular, influential, and high accuracy bull dies, leaving no semen. His EPDs are considerably better than his parents' EPDs. Semen from his clone has just hit the market and you are deciding whether to use it. Which EPDs do you use: the progenitor's or the clone's? You know the progenitor's EPDs reflect the clone's genetic merit, so you try a few straws. However, most breeders recognized that the clone's EPDs (currently equal to the progenitor's mid-parent EPDs) are what will influence his progeny's EPDs, so they decide to wait until the clone's EPDs have improved. The clone produces 20 progeny in his first year and you sell your bulls knowing their EPDs are lower than if they had been sired by the progenitor. Your only consolation is that the EPDs of the daughters you kept will eventually rise if the clone produces enough progeny for his EPDs to converge to the progenitors'.

To be theoretically correct and avoid the problems in the example, some genetic service providers fit clones as genetic identicals. There is a complicated way to fit this directly, but there is a very simple way to achieve the same result: within the genetic evaluation, assign all identical individuals the same ID (i.e., that of the progenitor), as if all the records and progeny of the clones were produced by the progenitor. In taking this approach it is recommended that clones retain their unique identities within the registration system so that, if we ever have enough data to make it feasible to estimate the degree, if any, to which clones differ in breeding value, we will have the information needed to estimate it.

It has been pointed out that, if all identicals are genotyped, the problem described above almost vanishes with current genomic evaluation procedures. Nonetheless, assigning all identical individuals a common ID for genetic evaluation will still be simpler and more accurate. The example above assumed the clone was not genotyped to illustrate the point.

The above discussion is the basis for a recent addition to the BIF Guidelines[1]:

"There are instances where genetically identical animals are in the pedigree (i.e. identical twins and clones). BIF recommends that, where genetically identical animals exist in the pedigree, for purposes of routine genetic evaluation, each set of genetically identical individuals is assigned a common identifier, so they have identical EPDs. Periodic test runs with the genetic identicals individually identified and the differences between them evaluated would be prudent. BIF recommends that genetically identical individuals should be assigned different permanent identification numbers." which appears in the section on "Expected Progeny Differences".

Gene Edited Animals

Gene editing is a process that allows specific modifications to be made to the genomic sequence of embryos or gametes resulting in animals that can transmit the desired modifications to their descendants. It is mentioned here because most approaches to gene editing involve manipulation and transfer of the embryo, but we leave description of the details and variations of it to others. [24] Currently, most, if not all, gene edited cattle are produced by either IVF or NT. Consequently, their records are implicitly excluded from genetic evaluations.

However, eventually descendants of gene edited animals will enter genetic evaluations. If gene editing only introduced the polled mutation into a breed that is mostly horned, it would probably have no effect on genetic evaluation. At the other extreme, using gene editing to inactivate the myostatin gene could wreak havoc on genetic evaluations of close relatives on both sides of the gene editing event for most traits currently evaluated. Other uses of gene editing would likely fall along a continuum between these extremes.

Gene editing directly violates fundamental assumptions of traditional (non-genomic) genetic evaluation. Fortunately, it is probably much easier to accommodate in genomic evaluation models. However, there are many different genomic models and the ways in which they could accommodate gene editing are likely to differ. This may be a challenging problem when it eventually materializes as a problem.

Records Produced by AI or Natural Service Progeny of Donors Subsequent to Superovulation

Non-ET (AI or natural service) calves whose dams had been previously superovulated weighed 2.2±0.4 lb more at birth than non-ET calves whose dams had not been previously superovulated[51], although many of those donors had been superovulated numerous times. Superovulating donors repeatedly predisposes them to obesity and may raise their tailhead, thicken their crest and make them appear generally "coarse" (Thallman, personal observation). The effect of superovulating a cow only once is less. Whether, and/or under what circumstances, records of natural calves produced subsequent to superovulation are suitable for inclusion in genetic evaluation requires further investigation. Records for reproductive traits collected subsequent to superovulation are not suitable for use in genetic evaluation.

Maternal Effect of Donor Cow

Previous models[47][48][49][50][51] to include MOET records for maternal traits in genetic analysis have been based on the over-simplified assumption that the recipient is the sole source of maternal effects. This approach is clearly superior to assigning the maternal effect solely to the donor, but it may be suboptimal. A better approach is likely to be to separately estimate the variances of the maternal effect of the recipient and the (presumably much smaller) maternal effect of the donor from appropriate field data. There are several reasons for this assertion:

It is not implausible that the early oviductal and uterine environment of the donor affects the subsequent development and phenotypes of the resulting animal, given the discussion about LOS and abnormal clone syndrome (ACS). Furthermore, the ovum cytoplasm is filled with mRNA transcribed from the maternal genome that guides the embryo through the first several cell divisions and may have effects beyond that.

Effects of gametic imprinting and X-chromosomes are not fit in current genetic evaluations of beef cattle. To the extent that they are important for a trait, these mechanisms are accounted for by the direct additive, maternal additive, and residual effects in the model. Based on similarity of design and relationship matrices, the majority of these mechanisms may be allocated to the maternal effect. Gametic imprinting captured by the maternal effect (because of imprinting not being fit in the model) has been proposed as a potential contribution to the negative genetic correlation that has long perplexed animal breeders. These effects are genetic, so in MOET, that would be the maternal effect of the donor, not of the recipient. In Brangus and Simbrah, gametic imprinting was estimated to account for 4.7 and 6.9% of phenotypic variance for birth weight in male and female calves, respectively[51].

Consequently, an improvement to the above model for MOET records may be to allocate the maternal effect between the recipient and donor, with the proportions estimated from field data. Nonetheless, challenges in implementing this refinement should not impede implementation of the current recommendations.

Recommendations of Current BIF Guidelines on Embryo Transfer

Please see http://guidelines.beefimprovement.org/index.php/Embryo_Transfer_(ET):_Data_Collection_And_Utilization for recommendations that reflect future updates. The recommendations in effect at the time of this presentation are:

"BIF recommends that observations from animals resulting from MOET, for traits that do not have maternal effects, be used in genetic evaluations provided any preferential treatment, if given, is accounted for by assigning an appropriate contemporary group code.

BIF recommends that observations from animals resulting from MOET, for traits that have maternal effects, be used in genetic evaluations as long as the recipient dams' ages (heifer, 1st parity, or multiparity) and approximate breed compositions are available, and any preferential treatment, if given, is accounted for by contemporary grouping.

BIF recommends use of recipient cows with known pedigrees well-tied to the genetic evaluation as being preferable to recipients with unknown pedigree and no previous calves with records in the genetic evaluation. Where this is not practical, each recipient dam should be assigned a unique identifier so occurrences of multiple ET calves with the same recipient are properly accounted for.

BIF recommends that embryo stage (1-9) and grade (1-3) [55] and whether frozen, split, sexed, or genotyped be recorded and submitted to breed association or other recording organization. BIF recommends that, when sufficient information becomes available, genetic evaluation models for MOET calves include effects of fresh versus frozen and of biopsied (sexed and/or genotyped) or not.

BIF recommends that records of animals produced by MOET should have separate contemporary group effects in the genetic evaluation from records of animals produced by AI or natural service. However, animals produced by MOET should be included in the same management code (as determined by the breeder) as animals not produced by MOET (including AI or natural service calves) that were managed identically in the same group so their common environmental effect can be accounted for in future genetic evaluations. Major differences in age, breed, origin, etc. among recipients should also be accounted for in genetic evaluation models.

BIF recommends to not use phenotypic observations in genetic evaluation from animals resulting from In vitro Fertilization (IVF), Nuclear Transfer, or that are not explicitly known to have resulted from natural service, AI, or MOET in genetic evaluations. BIF recommends that observations on ET calves be recorded and submitted to breed association or other recording organization, along with the form of technology (as listed above or others not listed) used to produce the ET calves.

BIF recommends that for genetic evaluations of traits with maternal effects, that direct effects (breeding value, genomic effects, breed composition, heterosis, etc.) be assigned to the donor or natural dam, and maternal effects (breeding value, genomic effects, breed composition, heterosis, permanent environment, etc.) with the recipient dam.

BIF recommends that records for reproductive traits collected subsequent to superovulation not be used in genetic evaluation."

Conclusions

Embryo transfer is a valuable tool in the genetic improvement of beef cattle. Calves produced by ET comprise a disproportionately large share of ancestors of seedstock animals. Unfortunately, many ET cattle have been selected based on EPDs with unnecessarily low accuracy because their own records were excluded from the genetic evaluation. The Beef Improvement Federation recently adopted Guidelines recommending that records of most cattle produced by multiple ovulation embryo transfer be included in genetic evaluation. Unfortunately, despite important advantages leading to its rapidly increasing use, in vitro fertilization can cause large offspring syndrome, which renders records of cattle produced by it unsuitable for inclusion in genetic evaluations using current models. Actions by breeders, breed associations, and ET service providers that could potentially allow records of in vitro fertilized cattle to be included in future genetic evaluations are proposed.

Citations

Beef Improvement Federation. 2021. Guidelines for Uniform Beef Improvement Programs. http://guidelines.beefimprovement.org/index.php/Guidelines_for_Uniform_Beef_Improvement_Programs (Accessed 5/13/21)

Mapletoft, R. J. 2013. History and perspectives on bovine embryo transfer. Animal Reproduction 10(n3):168-173

Lamb, G. C., P. L. P. Fontes, and N. Oosthuizen. 2019. In vitro fertilization (IVF) versus multiple ovulation embryo transfer (MOET): Making the decision to use one or both. In: Applied Reproductive Strategies in Beef Cattle, Knoxville, TN. p 233-249. http://www.appliedreprostrategies.com/2019/documents/14-Lamb-C.pdf (accessed 11-11-2020)

Demetrio, D., C. Looney, H. Rees, and M. Werhman. 2020. Annual Report of the AETA Statistical Information Committee, American Embryo Transfer Assn. https://www.aeta.org/newsletter/wp-content/Up1Mawlqo2/2020/11/2019-AETA-Statistics-Survey-Results.pdf (accessed 5-14-2021)

Garcia, S. M., F. Morotti, F. L. B. Cavalieri, P. A. Lunardelli, A. O. Santos, C. M. B. Membrive, C. Castilho, R. Z. Puelker, J. O. F. Silva, A. F. Zangirolamo, and M. M. Seneda. 2020. Synchronization of stage of follicle development before OPU improves embryo production in cows with large antral follicle counts. Anim Reprod Sci 221:106601. doi: 10.1016/j.anireprosci.2020.106601

Earl, C. R., D. T. Armstrong, and B. J. Irvine. 1995. Juvenile in vitro fertilization-embryo transfer (JIVET): The in vitro production of viable embryos from oocytes obtained from gonadotrophin-stimulated juvenile calves and lambs. In: Australian Assn. of Animal Breeding and Genetics. p 94-96

Granleese, T., S. A. Clark, A. A. Swan, and J. H. J. van der Werf. 2017. Increased genetic gains in multi-trait sheep indices using female reproductive technologies combined with optimal contribution selection and genomic breeding values. Animal Production Science 57(10):1984-1992. doi: https://doi.org/10.1071/AN15440

Daly, J., H. Smith, H. A. McGrice, K. L. Kind, and W. van Wettere. 2020. Towards Improving the Outcomes of Assisted Reproductive Technologies of Cattle and Sheep, with Particular Focus on Recipient Management. Animals (Basel) 10(2)doi: 10.3390/ani10020293

Keefer, C. L. 2015. Artificial cloning of domestic animals. Proc Natl Acad Sci U S A 112(29):8874-8878. doi: 10.1073/pnas.1501718112

Lust, D., T. E. Lawrence, and J. Sperber. 2019. 105 Use of cloning in beef production - the WTAMU PrimeOne Project. Journal of Animal Science 97(Supplement_2):59-60. doi: 10.1093/jas/skz122.109

Willadsen, S. M. 1986. Nuclear transplantation in sheep embryos. Nature 320(6057):63-65. doi: 10.1038/320063a0

Bondioli, K. R., M. E. Westhusin, and C. R. Looney. 1990. Production of identical bovine offspring by nuclear transfer. Theriogenology 33(1):165-174. doi: https://doi.org/10.1016/0093-691X(90)90607-U

Robl, J. M., R. Prather, F. Barnes, W. Eyestone, D. Northey, B. Gilligan, and N. L. First. 1987. Nuclear transplantation in bovine embryos. Journal of animal science 64(2):642-647. (Article) doi: 10.2527/jas1987.642642x

Willadsen, S. M., R. E. Janzen, R. J. McAlister, B. F. Shea, G. Hamilton, and D. McDermand. 1991. The viability of late morulae and blastocysts produced by nuclear transplantation in cattle. Theriogenology 35(1):161-170. doi: https://doi.org/10.1016/0093-691X(91)90155-7

Gouveia, C., C. Huyser, D. Egli, and M. S. Pepper. 2020. Lessons Learned from Somatic Cell Nuclear Transfer. Int J Mol Sci 21(7)doi: 10.3390/ijms21072314

Wilmut, I., Y. Bai, and J. Taylor. 2015. Somatic cell nuclear transfer: origins, the present position and future opportunities. Philos Trans R Soc Lond B Biol Sci 370(1680):20140366. doi: 10.1098/rstb.2014.0366

Wilmut, I., A. E. Schnieke, J. McWhir, A. J. Kind, and K. H. S. Campbell. 1997. Viable offspring derived from fetal and adult mammalian cells. Nature 385(6619):810-813. doi: 10.1038/385810a0

Campbell, K. H. S., J. McWhir, W. A. Ritchie, and I. Wilmut. 1996. Sheep cloned by nuclear transfer from a cultured cell line. Nature 380(6569):64-66. doi: 10.1038/380064a0

Kato, Y., T. Tani, Y. Sotomaru, K. Kurokawa, J.-y. Kato, H. Doguchi, H. Yasue, and Y. Tsunoda. 1998. Eight Calves Cloned from Somatic Cells of a Single Adult. Science 282(5396):2095-2098. doi: 10.1126/science.282.5396.2095

Oback, B., A. T. Wiersema, P. Gaynor, G. Laible, F. C. Tucker, J. E. Oliver, A. L. Miller, H. E. Troskie, K. L. Wilson, J. T. Forsyth, M. C. Berg, K. Cockrem, V. McMillan, H. R. Tervit, And D. N. Wells. 2003. Cloned Cattle Derived from a Novel Zona-Free Embryo Reconstruction System. Cloning and Stem Cells 5(1):3-12. doi: 10.1089/153623003321512111

Gerger, R. P. C., R. Rossetto, E. S. Ribeiro, I. Ortigari, F. C. Zago, L. H. Aguiar, U. M. Costa, R. F. F. Lopes, C. E. Ambrósio, M. A. Miglino, J. L. Rodrigues, F. Forell, L. R. Bertolini, and M. Bertolini. 2017. Impact of cumulative gain in expertise on the efficiency of handmade cloning in cattle. Theriogenology 95:24-32. doi: https://doi.org/10.1016/j.theriogenology.2017.02.025

Rutherford, B. 2014. Will Crossing Two Clones From Prime, YG1 Carcasses Produce Similar Offspring? Beef Magazine. https://www.beefmagazine.com/beef-quality/will-crossing-two-clones-prime-yg1-carcasses-produce-similar-offspring

Maday, J. 2018. Ty Lawrence Pushes Beef Quality to the Limit. Drovers Journal. https://www.drovers.com/article/ty-lawrence-pushes-beef-quality-limit

Lee, K., K. Uh, and K. Farrell. 2020. Current progress of genome editing in livestock. Theriogenology 150:229-235. doi: 10.1016/j.theriogenology.2020.01.036

Van der Berg, J. P., G. A. Kleter, and E. J. Kok. 2019. Regulation and safety considerations of somatic cell nuclear transfer-cloned farm animals and their offspring used for food production. Theriogenology 135:85-93. doi: https://doi.org/10.1016/j.theriogenology.2019.06.001

Troxel, T. Embryo Transfer in Cattle. University of Arkansas Extension Bulletin:4 pp. https://www.uaex.edu/publications/PDF/FSA-3119.pdf

Bonilla, L., J. Block, A. C. Denicol, and P. J. Hansen. 2014. Consequences of transfer of an in vitro-produced embryo for the dam and resultant calf¹. Journal of dairy science 97(1):229-239. doi: 10.3168/jds.2013-6943

Spijkers, S., J. W. Lens, R. Schats, and C. B. Lambalk. 2017. Fresh and Frozen-Thawed Embryo Transfer Compared to Natural Conception: Differences in Perinatal Outcome. Gynecol Obstet Invest 82(6):538-546. doi: 10.1159/000468935

Zhang, J., M. Du, Z. Li, L. Wang, J. Hu, B. Zhao, Y. Feng, X. Chen, and L. Sun. 2018. Fresh versus frozen embryo transfer for full-term singleton birth: a retrospective cohort study. J Ovarian Res 11(1):59. doi: 10.1186/s13048-018-0432-x

Li, Y., C. G. Donnelly, and R. M. Rivera. 2019. Overgrowth Syndrome. Vet Clin North Am Food Anim Pract 35(2):265-276. doi: 10.1016/j.cvfa.2019.02.007

van Wagtendonk-de Leeuw, A. M., B. J. G. Aerts, and J. H. G. den Daas. 1998. Abnormal offspring following in vitro production of bovine preimplantation embryos: A field study. Theriogenology 49(5):883-894. doi: https://doi.org/10.1016/S0093-691X(98)00038-7

Behboodi, E., G.B. Anderson, R.H. BonDurant, S.L. Cargill, B.R. Kreuscher, J.F. Medrano and J.D. Murray. 1995. Birth of large calves that developed from in vitro-derived bovine embryos. Theriogenology v44 p227-232

Numabe T., Oikawa T., Kikuchi T. and Horiuchi T. 2000. Birth weight and birth rate of heavy calves conceived by transfer of in vitro or in vivo produced bovine embryos. Animal Reproduction Science, 64 (1-2), pp. 13-20

H. Jacobsen, M. Schmidt, P. Holm, P.T. Sangild, G. Vajta, T. Greve, H. Callesen. 2000. Body dimensions and birth and organ weights of calves derived from in vitro produced embryos cultured with or without serum and oviduct epithelium cells. Theriogenology, v53, Issue 9 p1761-1769. ISSN 0093-691X. https://doi.org/10.1016/S0093-691X(00)00312-5

Luiz Sergio Almeida Camargo, Celio Freitas, Wanderlei Ferreira de Sa, Ademir de Moraes Ferreira, Raquel Varela Serapiao, João Henrique Moreira Viana. 2010. Gestation length, birth weight and offspring gender ratio of in vitro-produced Gyr (Bos indicus) cattle embryos/ Animal Reproduction Science. Volume 120, Issues 1–4, p10-15. ISSN 0378-4320. https://doi.org/10.1016/j.anireprosci.2010.02.013

Wells, D. N., J. T. Forsyth, V. McMillan, And B. Oback. 2004. Review: The Health of Somatic Cell Cloned Cattle and Their Offspring. Cloning and Stem Cells 6(2):101-110. doi: 10.1089/1536230041372300

Sinclair, K. D., T. G. McEvoy, C. Carolan, E. K. Maxfield, C. A. Maltin, L. E. Young, I. Wilmut, J. J. Robinson, and P. J. Broadbent. 1998. Conceptus growth and development following in vitro culture of ovine embryos in media supplemented with bovine sera. Theriogenology 49(1):218. doi: https://doi.org/10.1016/S0093-691X(98)90571-4

Young, L. E., C. G. Guttierez, S. C. Butterwith, J. J. Robinson, P. J. Broadbent, T. G. McEvoy, I. Wilmut, and K. D. Sinclair. 1999. Altered IGF binding protein expression is associated with large offspring syndrome in fetal sheep. Theriogenology 51(1):196. doi: https://doi.org/10.1016/S0093-691X(99)91755-7

McEvoy, T. G., K. D. Sinclair, P. J. Broadbent, K. L. Goodhand, and J. J. Robinson. 1999. Post-natal growth and development of Simmental calves derived from in vivo or in vitro embryos. Reproduction, Fertility and Development 10(6):459-464. doi: https://doi.org/10.1071/RD98126

Barker, D. J. P. 1995. The fetal and infant origins of disease. European Journal of Clinical Investigation 25(7):457-463. doi: 10.1111/j.1365-2362.1995.tb01730.x

Iwata H, Minami N, Imai H. Postnatal weight of calves derived from in vitro matured and in vitro fertilized embryos developed under various oxygen concentrations. Reprod Fertil Dev. 2000;12(7-8):391β396. doi:10.1071/rd00057

Yong-Soo Park, So-Seob Kim, Jae-Myeoung Kim, Hum-Dai Park, Myung-Dae Byun. 2005. The effects of duration of in vitro maturation of bovine oocytes on subsequent development, quality, and transfer of embryos. Theriogenology. Volume 64, Issue 1, Pages 123-134. ISSN 0093-691X. https://doi.org/10.1016/j.theriogenology.2004.11.012

Wilson, J. M., J. D. Williams, K. R. Bondioli, C. R. Looney, M. E. Westhusin, and D. F. McCalla. 1995. Comparison of birth weight and growth characteristics of bovine calves produced by nuclear transfer (cloning), embryo transfer and natural mating. Animal Reproduction Science 38(1):73-83. doi: https://doi.org/10.1016/0378-4320(94)01353-N

Chavatte-Palmer, P., R. S. F. Lee, S. Camous, N. Le Cleac'h, H. Jammes, and M. Guillomot. 2011. The placenta of bovine clones. Acta Scientiae Veterinariae 39(SUPPL. 1):s227-s242

Hill, J. R. 2014. Incidence of Abnormal Offspring from Cloning and Other Assisted Reproductive Technologies. Annual Review of Animal Biosciences 2(1):307-321. doi: 10.1146/annurev-animal-022513-114109

Panarace, M., J. I. Agüero, M. Garrote, G. Jauregui, A. Segovia, L. Cané, J. Gutiérrez, M. Marfil, F. Rigali, M. Pugliese, S. Young, J. Lagioia, C. Garnil, J. E. Forte Pontes, J. C. Ereno Junio, S. Mower, and M. Medina. 2007. How healthy are clones and their progeny: 5 years of field experience. Theriogenology 67(1):142-151. doi: https://doi.org/10.1016/j.theriogenology.2006.09.036

Thallman, R. M. 1988. Prediction of genetic values for weaning weight from field data on calves produced by embryo transfer, M.S. Thesis, Texas A&M University, College Station

Schaeffer, L. and Kennedy, B. 1989. Effects of embryo transfer in beef cattle on genetic evaluation methodology. Journal of Animal Science 67:2536-2543

Van Vleck, L. D. 1990. Alternative animal models with maternal effects and foster dams. Journal of Animal Science 68:4026-4038

Suárez MJ, Munilla S, Cantet RJ. 2015. Accounting for unknown foster dams in the genetic evaluation of embryo transfer progeny. J Anim Breed Genet. 2015;132(1):21:29. doi:10.1111/jbg.12121

Thallman, R. M., J. A. Dillon, J. O. Sanders, A. D. Herring, S. D. Kachman, and D. G. Riley. 2014. Large Effects on Birth Weight Follow Inheritance Pattern Consistent with Gametic Imprinting and X Chromosome. In: 10th World Congress on Genetics Applied to Livestock Production, Vancouver, BC Canada

Rivera, R. M. 2019. Consequences of assisted reproductive techniques on the embryonic epigenome in cattle. Reprod Fertil Dev 32(2):65-81. doi: 10.1071/RD19276

Kennedy, B. W., and L. R. Schaeffer. 1989. Genetic Evaluation Under an Animal Model When Identical Genotypes Are Represented in the Population. Journal of Animal Science 67(8):1946-1955. doi: 10.2527/jas1989.6781946x

Van Eenennaam, A. L. 2021. Gene Editing Today and in the Future. In: Annual Beef Improvement Federation Research Symposium, Des Moines, IA

Stringfellow, D.A. and M.D. Givens. 2010. Manual of international embryo transfer society (IETS). 4th ed. Champaign, Illinois: International Embryo Transfer Society

NOTES