Review Report

A practical review of assisted reproductive techniques in cattle. Part 2: recipient selection and embryo transfer

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Abstract

Nonsurgical transcervical embryo transfer in cattle is a technically demanding procedure that significantly influences pregnancy rates. This review outlines key determinants of success, including recipient selection, synchronization, and transfer technique for both in vivo-derived and in vitro-produced embryos. Appropriate recipient selection remains fundamental and should consider reproductive history, body condition, health status, temperament, and anatomical suitability. Synchronization protocols, including fixed-time embryo transfer programs, must balance physiological efficacy with practicality. Technical proficiency is a major controllable factor. Optimal outcomes are achieved by rapid, atraumatic deposition of the embryo in the cranial third of the uterine horn ipsilateral to the corpus luteum. Procedural difficulty, excessive manipulation, and incorrect deposition reduce pregnancy rates. Adjunct therapies, including nonsteroidal antiinflammatory drugs and hormonal strategies to increase circulating progesterone concentrations, may provide benefits in specific contexts but have variable outcomes.

Keywords: Embryo transfer, cattle, recipient selection, synchronization, ultrasonography

Introduction

Nonsurgical (transcervical) transfer of embryos into recipient cows is widely regarded by practitioners as one of the most technically difficult procedures in bovine reproduction. Whether transferring a conventional in vivo-derived (IVD) or in vitro-produced (IVP) embryo, the entire process, and successful establishment of pregnancy, largely depends on the technical proficiency of the practitioner relocating the embryo into a recipient female. Despite the first reported nonsurgical recovery attempts,[1] it was not until nearly 2 decades later that nonsurgical transfer techniques were fully developed during late 1970s and 1980s.[1-3] Nonetheless, ongoing surgical transfers provided the crucial information (e.g. embryo stage and location) necessary to achieve success without general anesthesia or a laparotomy, contributing to refinement of the nonsurgical technique.[3,4]

Although it initially appeared to be a straightforward process, embryo transfer (ET) proved far more complex than anticipated. Even highly experienced practitioners report the need for continual refinement of their technique. Numerous studies have demonstrated that technical skill has a substantial impact on pregnancy outcomes, suggesting that only experienced practitioners should conduct the procedure.[1,5-7] Interestingly, the influence of the ET technician/practitioner on successful posttransfer establishment of pregnancy appears more pronounced with frozen-thawed IVP embryos and those of lower morphological quality, emphasizing the value of technical proficiency.[6] Nonetheless, given the complexity of the IVP system, it is difficult to attribute success or failure to any single stage of the process. In addition to employing rigorous recipient selection protocols and optimal recipient management, the transfer procedure itself represents one of the few components directly controlled by the practitioner; therefore, mastering the technique is crucial for achieving optimal outcomes.

Recipient synchronization

The timing of ET was initially based on the interval after observed natural estrus, before availability of effective synchronization protocols.[8] Strategies for synchronization of recipient females for ET are fundamentally similar to those employed for estrus synchronization prior to artificial insemination, thus enabling fixed-time embryo transfer (FTET).[9]

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Citation: Clinical Theriogenology 2026, 18, 14225, http://dx.doi.org/10.58292/CT.v18.14225

Historically, the ideal window for transfer of a conventional/IVD embryo was into a recipient that had exhibited estrus 6.5-8.5 days prior to transfer.[10,11] Most synchrony programs therefore aim for the transfer of embryos recovered from donors that were in standing estrus on approximately the same day as the recipient, i.e. 7 days before transfer.[12,13] Even though most agree that the recipient should exhibit estrus from 36 hours before to 12 hours after the donor,[14,15] others have reported that no differences in pregnancy rates exist for recipients exhibiting estrus up to 24 hours after the donor in conventional ET programs.[16] Many practitioners report even greater recipient synchrony plasticity, and some even prefer recipients that express estrus up to 24-36 hours after the donor.[17] This variation may be related to the developmental stage at which embryos are collected and selected. Hence, unsurprisingly, practitioners have attempted to match embryo stage (and code) with the exact time of recipient estrus (unpublished data), although this may be difficult in large programs.

Although similar principles apply to IVP/IVF embryos, the interval from standing estrus to ovulation influences outcomes, depending on whether days 6, 7, or 8 recipients are used. Recipients of IVP embryos expressing standing estrus 8 or 7 days before transfer achieved higher pregnancy rates than those that were 6 days after estrus.[5,18] The preference for a slightly more advanced recipient (relative to those in multiple ovulation and embryo transfer [MOET]/IVD systems) reflects that IVP embryos are typically more developmentally advanced at transfer. Practitioners also report that breed and species differences must also be considered, as they also influence embryo development. For instance, *Bos indicus* embryos develop faster than their *Bos taurus* counterparts or Wagyu cattle in most IVP systems (unpublished data).

Fixed-time embryo transfer (FTET)

As with artificial insemination synchronization programs/protocols, there are a plethora of options to synchronize estrus for FTET. Choosing a suitable program needs to consider not just ovarian physiology and ovarian follicular dynamics but also what is practical. For instance, the added benefit of an additional handling and treatment for extensively managed *Bos indicus* recipients in hot climates may not necessarily outweigh the induced stress - a factor known to decrease pregnancy rates in ET recipients.[19-21] Alternatively, in intensive dairy systems, most would opt for a conventional OvSynch-based synchrony program, with or without an intravaginal device[22,23] and/or the addition of a Presynch strategy to improve response to the first gonadotropin releasing hormone (GnRH) in conventional OvSynch synchrony.[22,24,25]

In countries that allow estradiol-based products in food-producing animals, one could consider treating with estradiol benzoate (EB) and inserting a progesterone releasing device for 7-8 days, with prostaglandin at or prior to device removal,[9,26] with either estadiol cypionate at device removal or EB ~ 24 hours after removal.[27,28] Periovulatory treatment of estradiol esters may also enhance uterine receptivity and subsequent embryonic attachment,[29] a phenomena also recognized in recipient mares with a shorter preceding estrus period exhibiting lower posttransfer pregnancy rates and the necessary inclusion of estradiol for synchrony of anestrous mares.[30,31]

A newer protocol known as the '7 & 7 Synch' protocol (involves placing an intravaginal progesterone device for 14 days and prostaglandin treatment 7 days before initiating a GnRH-based synchronization program followed by GnRH) was compared to conventional 7-day 'CO-Synch' GnRH-based protocols, and was a promising alternative, particularly for postpartum beef cows.[32,33] The authors recommend readers familiarize themselves with the Beef Reproduction Task Force synchronization programs for artificial insemination when considering synchrony programs.[34] Regardless of the specific program used to facilitate FTET, expression and recognition of estrus are still key to identifying and using high-quality, fertile recipients, regardless of embryo type.[9] Examples of FTET programs for recipients (beef and dairy) are in Figure 1.

Recipient selection

When identifying a suitable recipient, one must consider several factors ranging from individual cow anatomy to herd-level characteristics such as lactation status, body condition, disease prevention and herd biosecurity. Other important considerations are ideally selecting on previous reproductive history (if possible), those that became pregnant early in the breeding season, calved early and raised strong, healthy calves. It is best to avoid cattle that failed to become pregnant that season (or previously), those that failed on a previous ET program, and purchased cattle with no known reproductive history. Heifers have the added advantage of no lactation or calves at foot, although have no proven reproductive history and may be difficult to transfer for the novice practitioner.

In general, it is recommended that recipients are at least 45-60 days after calving (the latter is recommended by most), cycling (i.e. palpable corpus luteum [CL] or regular recorded interestrus intervals in remote monitoring dairy systems), appropriate body condition (i.e. 2.5-3.5/5 scale), rising plane of nutrition for at least 6 weeks before and after transfer, having received appropriate disease prophylaxis, and having ideally only received no more than 1 or 2 unsuccessful transfers prior to reuse.[35] If heifers are used, it is best they fit the general requirements for replacement heifers prior to any breeding program; most importantly reproductive maturity assessed by weights and suitable reproductive tract scores.[15] Practitioners should also assess lactating cows with calves at foot for any injuries to the reproductive tract acquired during calving. Vaccination programs should also be followed as per usual for conventional cow-calf breeder herds, paying close attention to regional requirements, and vaccine type (i.e. modified live vaccines), with primary and secondary booster vaccinations treatments 30 days prior to breeding,[10,36] with potentially more plasticity with MLV treatment before AI or ET programs that otherwise thought.[37]

To identify a potential individual recipient, one must consider the 'usability' of the physical reproductive tract (i.e. cervical patency and uterine horn maneuverability), animal temperament, structural conformation and health assessments relevant to the recipient's ability to deliver, nourish and raise a healthy calf. Information available for potential recipients vary according to production system, i.e. intensive housed dairy systems with good records compared to extensive beef enterprises. In addition to resumption of cyclicity before initiating estrus synchronization, any prior evidence of uterine disease after calving (metritis or endometritis) can severely influence pregnancy rates (unpublished).

Physical attributes of the reproductive tract also influence a successful transfer and require years of practice and trial and

Citation: Clinical Theriogenology 2026, 18, 14225, http://dx.doi.org/10.58292/CT.v18.14225

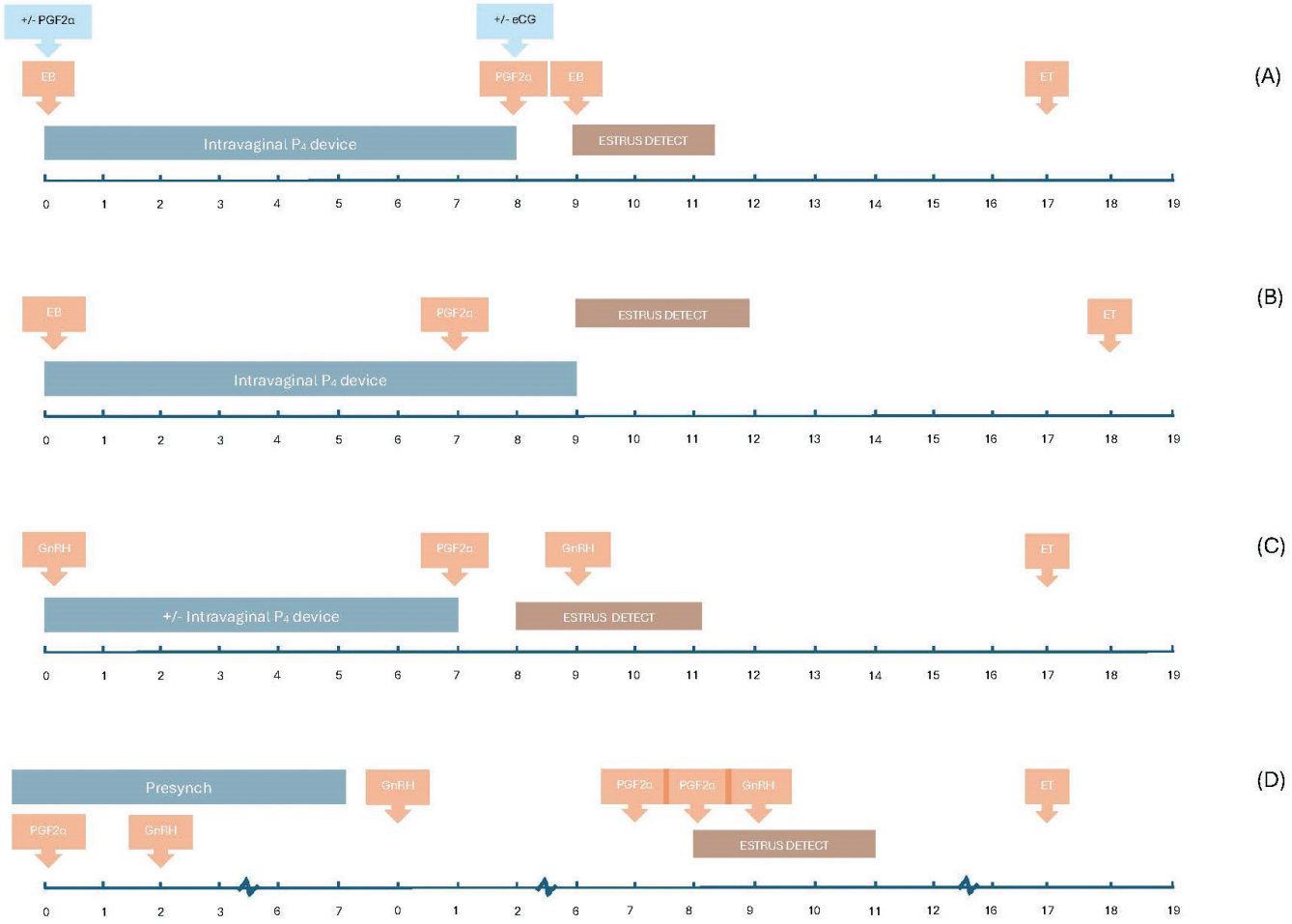

Figure 1. Various example protocols for synchronization of recipients for timed AI or ET in beef and dairy cattle; A. Example protocol where EB is used (not registered for use in North America), eCG is also optional; B. Program that relies on accurate estrus detection, despite synchronization; C. Example protocol suitable for lactating dairy cows with or without a progesterone releasing intravaginal device; and D. Example protocol based on the conventional 'OvSynch' with the addition of a PreSynch program (G6G) prior to the first GnRH of OvSynch

error before a suitable technique can be used on each unique tract. For instance, recipients with *Bos indicus* influence (i.e. Brahmans) and those crossbred to other animals (e.g. Brangus, Santa Gertrudis, Charbray etc.) can have a large and tortuous cervix that is extremely difficult to navigate.[38,39] Consequently, the authors occasionally use a cervical dilator (Minitube, Australia or IMV Technologies Global, Figure 5), in the extremely rare event when a cervix cannot be passed with a conventional ET gun and steel-tipped sheath or for those with an obvious sharp bend palpable in the cervix. Multiparous, postpartum adult lactating dairy cows or obese animals also may have a large dependent uterus that can be difficult to retract, making it difficult to advance the ET gun to the cranial portion of the uterine horn. This can again be extremely difficult for a novice practitioner to achieve correct deep uterine horn embryo placement.

General health and wellbeing are also important, particularly body condition and metabolic status. Body condition score is a crucial factor in resumption of estrus and ovulation postpartum[40] and inextricably implicated in establishment and maintenance of healthy pregnancies to term.[40,41] Structural conformation is crucial in the beef cow that is responsible for rearing the embryo-derived calf. Special consideration should be paid to not just musculoskeletal soundness but also udder conformation, as it can affect postnatal calf health.[42]

Temperament is an additional factor that cannot be under emphasized. Not only does excitability and movement make completing the procedure in an atraumatic fashion difficult, but cows with excitable temperament have a lower ET pregnancy rate than their less excitable counterparts.[43] The influence of behavior could mostly be the result of inability to complete the transfer atraumatically, as opposed to 'stress.' In most female mammals, disruption of reproductive function typically requires chronic stress exposure[44] whereas acute stressors are less likely to elicit sufficient cortisol release to impair hypothalamic-pituitary-gonadal axis function.[45] Gentle animal handling and good facilities are, however, vitally important to ensuring slow-stress handling and mitigating stress before and after ET, as cattle are handled over several weeks.

Equipment

Recommended equipment needed to perform the procedure is outlined (Figure 2).

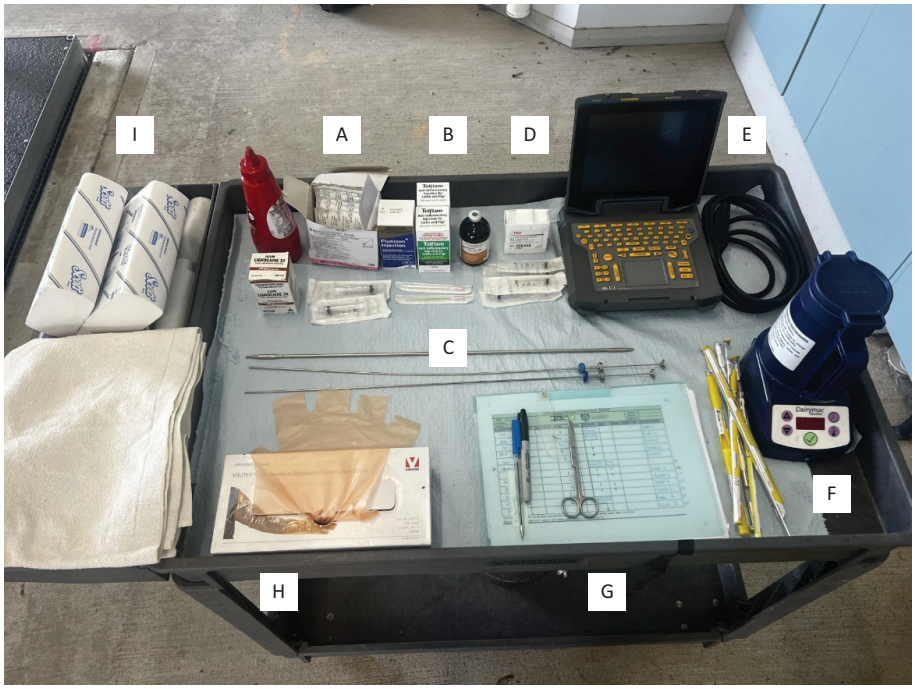

Figure 2. Image depicting the author's recommended setup for transfer of embryos into recipient cows; A. 18-gauge, 1.5-2 inch hypodermic needles and lidocaine for caudal epidural anaesthesia; B. medications including antiinflammatories and sedation (acepromazine maleate) if indicated; C. deep seated ET guns and cervical dilator (if indicated); D. sterile chemises to go over loaded ET gun; E. High-quality transrectal ultrasonographic machine; F. Electric thawer; G. Records; H. rectal sleeves/gloves with finger ends removed and powder-free examination gloves to place over the top to improve palpation sensitivity; and I. Paper towels and towels to clean the recipients vulva prior to transfer

Reproductive tract assessment and characteristics of the corpus luteum

Transrectal palpation and ultrasonography

Most authors agree that using ultrasonography increases sensitivity to correctly identify a CL.[46-48] Furthermore, there appears to be limited influence of the visible characteristics of the CL at ET on pregnancy rates, so long as estrus has occurred within a reasonable time frame.[49,50] Perhaps this is overinterpretation, as ultrasonography has improved over time. Earlier studies suggested that the cavitation within a CL negatively influenced pregnancy rates in ET recipients (and was colloquially accepted throughout the ET community), although more recent reports now disagree[49-51] by documenting improved pregnancy rates and higher progesterone concentrations for CL with cavities.[50-52] It is plausible some of the previous disagreement is due to misdiagnosis of 'luteal cysts' as a cavitary CL.[53] Differentiating the 2 structures can be difficult and there are therefore disputes regarding clear definitions.[53] Central cavities are detectable from 3-5 days after estrus, reach their maximum diameter on days 6-12 and most decrease in size and disappear by days 16-20.[54-56] Figure 3 has an example of this when a recipient was reexamined at subsequent pregnancy diagnosis at ~ 30 days of pregnancy.

The overall size (therefore volume) of luteal issue has been postulated to be associated with pregnancy rates after embryo transfer.[57] However, more recent articles suggested that size may have limited overall influence on pregnancy rates than otherwise assumed.[10,49,57,58] Given these contradictory findings, it is worth emphasizing that the suitability of a potential ET recipient is likely better focused on accurately recognizing and recording recipients in standing estrus and identifying an adequate CL, regardless of morphological appearance, using standard B-mode ultrasonography.[9,49,58] There are recent advancements in using Doppler ultrasonography to facilitate a deeper assessment of the CL prior to embryo transfer. The theory is that CL with improved vascularity and perfusion (measured by increased intensity of power Doppler or color flow Doppler area) may deliver higher progesterone concentrations more efficiently and are associated with better reproductive success.[27,59-62] However, many articles cited required retrospective analysis of imagery to determine objective cut-off values for color blood flow area or pixel intensity. Others have described a scoring system from 1-4 that may improve real-time assessment and facilitate application.[59] Integrating software into ultrasonographic machines can reduce or eliminate subjectivity, and improve practicality. Assessing blood flow area measured by color Doppler (Figure 4) may be more predictive of pregnancy outcome than CL size alone.[27] Overall these are interesting findings as they relate to the previously aforementioned notion that vascularization and perfusion (i.e. 'CL efficiency'), as opposed to luteal diameter, are important factors for selecting a 'good' CL.[27] In our experience, one needs to apply the correct settings to get a good image and that may be too time-consuming in large-scale transfer programs. It is noteworthy that fixed-time programs and assessing CL quality does not outweigh the importance of correctly identifying and using recipients that were detected in estrus.

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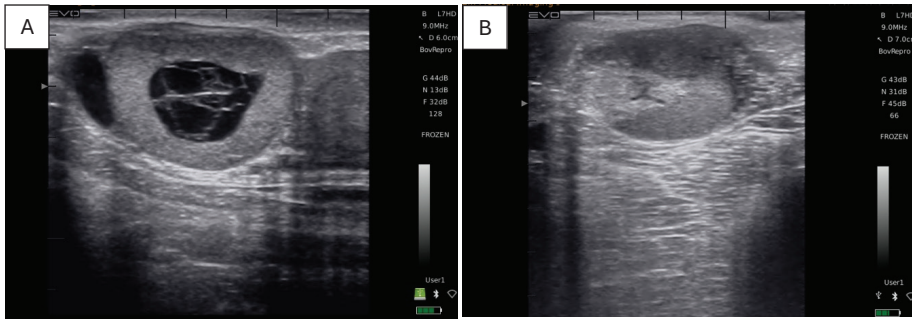

Figure 3. Series of images obtained via transrectal palpation and ultrasonography; A. CL with a central cavity recognized in a day 7 recipient prior to embryo transfer; and B. Same CL, 3 weeks later

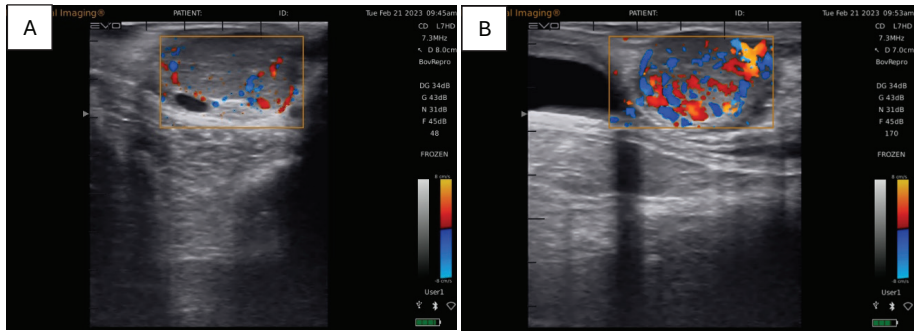

Figure 4. Images of various corpora lutea obtained via transrectal ultrasonography assisted by color flow Doppler imaging; A. Example of a CL considered to have limited blood flow; and B. CL with substantial blood flow

Transfer technique and site of embryo deposition

Practitioner must deposit the embryo as far cranial as possible in the uterine horn ipsilateral to the identified CL from an induced ovulation 7-8 days before.[22] Transfer of the embryo in the uterine horn contralateral to the CL results in severely reduced pregnancy rates[63-66] and naturally occurring contralateral pregnancies occur in < 1% of cattle.[67] Most would agree that deposition of the embryo to the cranial third of the uterine horn ensures the highest likelihood of pregnancy, a finding established during the experiments that facilitated the transition to nonsurgical techniques.[1,68] Unsurprisingly, at day 7 after ovulation, the cranial portion of the ipsilateral uterine horn is also where most embryos are located.[69,70] Despite reports that depth of deposition along the ipsilateral uterine horn alone has limited statistical relevance, this could be consequent to technician effect and/or smaller populations.[1,71] Additionally, others report a potential interaction between embryo grade/quality and depth of deposition on pregnancy rates, with lower-quality (Grades 2-3) embryos potentially having significant reduction in pregnancy rate when the cranial third of the horn cannot be reached.[72] It is physiologically reasonable to replace the embryo back to its original location, and where they are known to instigate the Type I interferon-response and upregulation of endometrial transcripts necessary to transform uterine luminal metabolites and maintain pregnancy.[73] Unlike in mares, there are only few reports of uterine embryonic migration in singleton bovine pregnancies[74] and therefore the embryo must be placed **'where it came from, and where it wants to be'.**

Anatomical variation among individuals, including breed, body condition, postpartum status and parturition history can complicate transfer. A heavy, dependent uterus, particularly in lactating dairy or over-conditioned beef cows, may limit access to cranial uterine horn. In such cases, a balance must be struck between achieving optimal deposition depth and avoiding endometrial trauma that may induce prostaglandin release. Even mild endometrial trauma could cause bleeding and activation of complement proteins that could be potentially embryocidal and cause release of luteolytic prostaglandin.[14] Although the time taken for cervical passage appears to have limited influence on overall pregnancy rates, time spent within the uterus certainly does.[1,68,75] However, more recent reports suggested otherwise, indicating difficult cervical passage could influence pregnancy establishment, overall implying that a 'difficult' transfer (regardless of location difficulty) indeed influences outcomes.[68,75] Therefore, excessive uterine and/or cervical manipulation and prolonged restraint is undoubtedly counterproductive.[68,75] Pregnancy rates can be reduced significantly if it takes > 1 minute to pass the uterine horn greater curvature.[76] Minimizing manipulation and ensuring rapid, atraumatic placement of the embryo in the cranial ipsilateral horn are therefore critical to maximizing success,[71] i.e. **'put it back gently and swiftly from where it came from.'**

Procedure

In summary, the procedure for transfer of a freshly loaded or thawed/warmed embryo is described herein. It is noteworthy that no 2 ET procedures are identical, and individual practitioners often employ slight variations in technique while adhering to the fundamental principles of nonsurgical

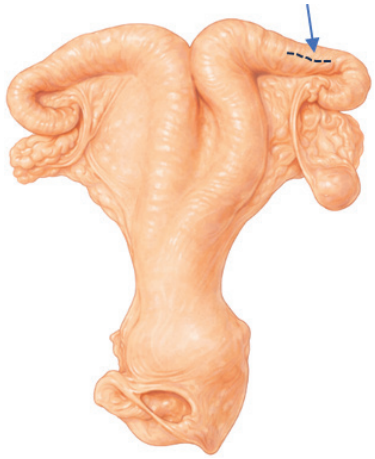

Figure 5. Illustration detailing recommended location of embryo deposition in nonsurgical (transcervical) embryo transfer in cattle

transfer. Once the recipient is calmly restrained in the chute, identification is confirmed and estrus detection aids, where used, are assessed against recorded data. A lidocaine caudal epidural (lidocaine hydrochloride, maximum 1 ml/100 kg body weight) is given in the sacrococcygeal space, and the practitioner examines the reproductive tract via transrectal palpation, ideally limiting excessive uterine manipulation. The CL from the previous ovulation is identified and assessed as previously described and the uterine horns are gently retracted to ensure maneuvrability. Vulva is wiped clean (generally without disinfectant) using paper towel or a scraper fashioned from a square section of plastic or from the back of a CIDR or Cue-Mate applicator. The ET gun (Deep chamber ET Gun, IMV Technologies) with sterile lateral portal ET sheath (single or multipack, 3 mm ET Sheath, IMV Technologies; Transfit Lateral ET Sheath 0.25 ml straw, Minitube) inside a sterile sanitary chemise (A.I./E.T. Sanitary sheaths, 24 inch, IMV Technologies) is then passed to the technician/practitioner. Although some practitioners part the vulvar lips prior to rectal insertion, most maintain the arm in situ following identification of CL to avoid air introduction that is difficult to resolve after epidural treatment. The assistant then parts the vulva widely, and the transfer gun is introduced with the tip pointed ~ 45 ° upwards and advanced toward the external cervical os. Care must be taken to prevent the sanitary sheath from catching vaginal folds; that can be minimized by adequate vulvar separation or application of a small amount of sterile lubricant to the chemise tip. Once the external cervical os is reached, the chemise is then broken and pulled back toward the plunger. Cervix is then traversed softly, 1 ring at a time. In some cattle (particularly recipients with *Bos indicus* influence), the cervix has bends or blind endings that require gentle manipulation to facilitate passage. In those with an obvious or severe bend, or extremely narrow canal, a cervical dilator (Figure 6) may be considered as a last resort.

Following cervical passage, the catheter tip may briefly rest within the uterine body. From this point, gentle uterine manipulation is critical, with only minimal backward hand pressure applied to facilitate advancement. Uterus should be unfolded, lifted, or straightened via transrectal palpation to facilitate controlled advancement of the catheter, rather than advancing (or threading) the uterus over the tip of the gun. Depending on which horn is to be traversed, various strategies are employed. Direction into the ipsilateral uterine horn is achieved by repositioning the tract (e.g. lateral displacement or complete rotation) to enter and progress through the uterine horn of interest.

Anatomical variation (e.g. short internal bifurcation) may impede progression despite correct positioning, requiring further adjustment of uterine orientation. The ET gun should be advanced as far cranially as possible, ideally beyond the greater curvature of the uterine horn (which is always attempted by authors). This is achieved by elevating or repositioning (by lifting underneath) the uterine horn while maintaining minimal manipulation. Direct contact with the uterine horn distal to the catheter tip should be avoided where possible, and only gentle finger pressure should be applied. Manipulation of the broad ligament may also be helpful to avoid direct uterine pressure.

At the site of deposition, uterine horn is stabilized behind the catheter tip, embryo is expelled with gentle plunger pressure, and the gun is withdrawn promptly. Minimizing postprocedural disturbance is recommended. Although techniques vary among practitioners (who all have their own repertoire for maneuverers for each unique tract encountered), adherence to these basic principles supports consistent and atraumatic embryo transfer.

Strategies to maximize pregnancy rates

Beyond excellent general recipient management principles (i.e. nutrition, vaccination, etc.), attentive recipient and embryo selection, gentle cattle handling and mastering the 'fast and atraumatic' transfer technique, practitioners and researchers have sought to investigate various strategies to improve overall pregnancy rates. It still remains an enigma as to why the average pregnancy rates from conventional and in vitro embryos often do not surpass those through natural mating,[8] considering that most pregnancy attrition occurs in the first week after fertilization - a developmental timepoint that embryo transfer should theoretically circumvnavigate.[8,77] Some therapeutic strategies include the use of antiinflammatories, progesterone/progestin products, luteotrophs, and ovulating induction agents.

Nonsteroidal antiinflammatories

Transcervical intrauterine ET induces a transient release of prostaglandin $F_{2\alpha}$ from the endometrium in cattle and horses.[78] It is therefore unsurprising that the use of nonsteroidal antiinflammatories (NSAIDs) at ET has been widely investigated.[43,75,79-84] A critical review[75] investigated 16 trials from 9 publications and surmised that the evidence does suggest NSAIDs improve outcomes in recipients, particularly in those with difficult transcervical passage. However, there were not enough data to fully discern whether NSAIDs are best given before or after ET. Although a recent publication involving the use of tolfenamic acid had no overall difference in pregnancy rates, analysis of subgroups revealed a beneficial use in animals of low body condition, in heifers with a smaller CL and recipients receiving IVF embryos not having reached the expanded blastocyst stage.[80] Further, an NSAID at transfer may improve success rates in cattle excitable at embryo transfer.[43] Although its routine use in large-scale recipient programs may be difficult to justify, it may be warranted for high-value embryos or in smaller programs involving recipients with

Citation: Clinical Theriogenology 2026, 18, 14225, http://dx.doi.org/10.58292/CT.v18.14225

Figure 6. Cervical dilator with larger ball-tip (above); best used for improving cervical passage while flushing embryo donors and smooth end (below); best used for recipients if necessary as a last resort

challenging reproductive anatomy or temperament. In mares, an NSAID may also improve after transfer pregnancy rates.[85]

Induction of accessory CL and increasing circulating progesterone

Practitioners and researchers have previously explored the use of hormones and treatments as an aid to increase progesterone concentrations in ET recipients. Intuitively, this comes from the historical notion that supplemental progesterone (exogenous or endogenous) could reduce embryonic loss and improve pregnancy rates in subfertile animals.[86-88] Many studies have explored using hormones to increase endogenous progesterone. However, caution is indicated as the continual effect of increasing progesterone beyond > 1 ng/ml is questionable. Supplementation with exogenous progesterone itself is variable and controversial with either intravaginal device (CIDR, PRID, or other) reinsertion on the day of transfer or injectable options having limited to no influence on overall pregnancy rates.[87-89] However, consequent to the ongoing dynamics of follicular waves throughout early pregnancy, there is opportunity to facilitate induction of additional/supplementary CL as a strategy to maintain and improve endogenous progesterone support. Interestingly, this is similar to what happens as the natural 'fail-safe' mechanism for pregnancy maintenance in mares with development of endometrial cups, production of eCG and accessory CL formation to extend the ovarian progesterone production prior to a luteoplacental shift in pregnancy maintenance.[90]

Benefits of hormonal support for ET were assessed via 2 large-scale meta-analyses.[87,91] Improvements were evident (4% with endogenous progesterone and 8% for GnRH or hCG treatments). However, further scrutiny into animal populations and timing of treatment provided more detail on the scale of this improvement. It is noteworthy that concentrations beyond an arbitrary threshold are not necessarily additionally beneficial and minimum progesterone concentrations to support pregnancy may be lower than what was assumed.[10,36,92-94]

Human chorionic gonadotropin

Although human chorionic gonadotropin (hCG) improved endogenous progesterone concentrations in dairy cattle and is likely beneficial for improving overall fertility, its benefit for consistently improving pregnancy rates in ET is still debated.[87] In dairy recipients, hCG treatment at transfer increased circulating progesterone concentrations but not overall transfer pregnancy rates.[95] However, others reported different findings in beef cattle, especially when hCG was given 3-4 days before transfer[96] or at transfer with day 5.5-8.5 recipients of various breeds.[97] In 1 study, hCG was beneficial in beef heifers given at day 5 after estrus; it increased progesterone concentrations and conceptus size.[98] Similar findings were reported in other beef breeds[99] and dairy-beef crossbred recipients[100] that received it 1 or 2 days prior to transfer (days 5 or 6 after estrus) respectively, possibly due to an increase in overall luteal area consequent to induction of accessory CL and/or luteal hyperplasia/hypertrophy.[100,101] Treatment timing is important, with most agreeing its use is best within the first week after ovulation and before transfer[101] or at transfer[97] but not necessarily when used earlier, for instance, at ovulation or in combination with other synchronization strategies prior to estrus as it may be counterproductive.[102] It is important to scrutinize these studies (namely population sizes and animal demographics); the huge variables associated with ET outcomes (especially IVP embryos) requires large datasets to detect real differences.

Gonadotropin releasing hormone

In a recent meta-analysis, similar to hCG, GnRH treatment 5-7 days after ovulation increased pregnancy rates, although only in cattle subpopulations with inherently low fertility, i.e. high-producing lactating dairy cows under metabolic stress.[91] Although this review identified no major improvement in reducing pregnancy losses after ET, treatment of GnRH could cause formation of an accessory CL, increase peripheral progesterone concentrations and reduce pregnancy losses in heifers that received an expanded blastocyst IVF embryo.[103] GnRH is a relatively low-cost option and could be considered in some ET programs, noting its applicability to only specific-animal demographics.

Equine chorionic gonadotropin

Inclusion of equine chorionic gonadotropin (eCG) in common synchronization protocols improved recipient utilization and overall pregnancy rates in FTET programs.[104,105] Justification for its usage appears to be related to increased growth of the preovulatory dominant follicle/s and increased plasma progesterone concentrations after ovulation.[104] Data extrapolated from FTAI trials suggested that eCG is most effective in improving fertility in animals with lower body condition score,[106] those early postpartum,[107] or under other physiological stressors.[108,109] The use of eCG at embryo transfer however (i.e. 7 days after ovulation) appeared to have no significant benefit[110] and was best given at intravaginal device

removal (prior to ovulation) to improve luteal area and peripheral progesterone concentrations.[96] However, it is no replacement for adequate nutrition and good animal management.

Surgical transfer

Although most (if not all) ET procedures are now performed using nonsurgical transcervical techniques, surgical transfer may still be indicated in rare cases, such as when cervical passage is not possible or in research settings requiring oviductal transfer of early-stage embryos.[111,112]

Surgical embryo transfer technique

Following identification (via transrectal palpation) of the ovary having the CL, recipients are sedated using either intravenous acepromazine (0.03 mg/kg) given prior to restraint or intravenous xylazine (0.02 mg/kg) once restrained, although the latter may induce uterine contractility and limit uterine exteriorization, and therefore is not recommended by some authors.[113] The surgical site, located caudal to the paralumbar fossa, is clipped and aseptically prepared. Regional anaesthesia is applied using a line block across the anticipated surgical site using lidocaine hydrochloride. Although most clinicians would use an 'inverted L' or 'distal/proximal paravertebral' block for a paralumbar fossa/flank approach in most standing abdominal surgeries,[114] the incision to access the cranial portion of the uterus and ovary is more caudal than a traditional flank incision and is best made diagonally (Figure 7).

The upper diagonal branch of the external abdominal oblique is incised, followed by the internal abdominal oblique and then manual separation of the transverse bands of the transversus abdominus until the peritoneum is encountered and gently punctured (either with blunt force or by tenting with forceps and making a gentle incision with a scalpel).[113] On entering the abdomen, the hand is directed caudodorsally to gently grasp the ovary and the previously identified CL is confirmed. The uterine horn is then gently retracted to the abdominal incision, and a small entry hole is made through the uterine perimetrium into the uterine lumen using a blunt (or sharp) 18 or 19-gauge needle. Using a preloaded tom-cat catheter, the embryo is deposited into the cranial aspect of the uterine horn. Prior to deposition the catheter is gently moved back and forth to confirm entry into the lumen as opposed to endometrium or myometrium. After deposition, the uterus is placed back within the abdomen and the abdomen is closed routinely with suture pattern of choice. Typically, prophylactic antibiotics are not generally indicated unless abdominal contamination is suspected; posttransfer management of recipients is generally the same as nonsurgical candidates.

Novel transfer techniques and devices

Given the challenges associated with transitioning from surgical to nonsurgical embryo transfer techniques, a range of alternative methods and devices have been investigated. A transvaginal endoscopic approach for oviductal transfer was explored in the late 1990s; however, it did not gain widespread adoption consequent to its invasiveness, procedural duration, and limited field applicability.[111,115] Devices adapted from deep uterine horn insemination, such as the Ghent device,[116] have also supposedly been evaluated, although outcomes appear comparable to those achieved by skilled practitioners.

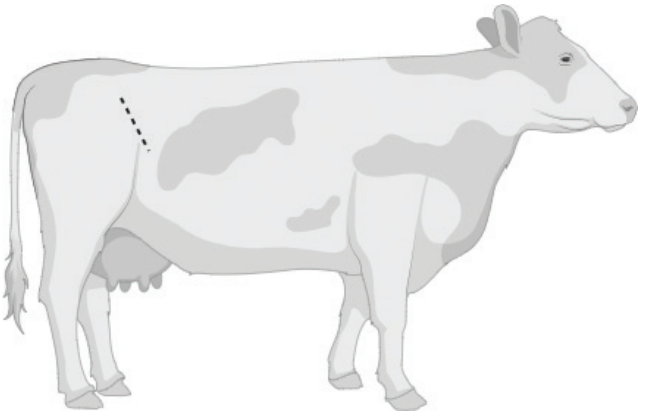

Figure 7. Recommended location for incision to exteriorise uterine horn and ovary for surgical embryo transfer

Recent advances in engineering have renewed interest in device-assisted transfer systems. For example, the XtremET device (AXCE, France)[117] is designed to facilitate deep uterine deposition without the need for transrectal guidance, using a flexible catheter that conforms to uterine anatomy. Although such technologies may offer practical advantages, their clinical efficacy remains to be fully established. With increasing global access to bovine IVF (including developing nations) and emerging technologies such as gene editing, limitations in skilled labor and cost remain major barriers to widespread adoption of embryo transfer. Accordingly, there is growing interest in technologies that reduce the bottleneck of skilled operator dependency. However, at present, successful ET outcomes remain highly reliant on experienced practitioners with advanced palpation skills.

Conclusion

The review aimed to synthesize current knowledge on recipient selection, synchronization strategies, and technical aspects of ET in cattle, with emphases on factors that can be practically controlled to optimize pregnancy outcomes. Despite rapid advances in embryo production, nonsurgical transcervical transfer of the embryo is still a crucial, and unavoidable final step to the ET process that has not changed for nearly 30-40 years. This process still hinges on competent practitioners with exceptional palpation skills and technical ability. Pregnancy rates achieved through ET (particularly those derived from MOET) remain similar to those encountered after natural mating, suggesting that the ovum pickup (OPU), IVF and/or MOET/ET process does not necessarily circumnavigate natural pregnancy attrition.[16,77,86] From a holistic standpoint, this implies that the process is somewhat unrefined. Although many factors ultimately influence the successful establishment of pregnancy, the effect of technician on pregnancy rates is a well-established variable that can be controlled in most systems.[5,6] Many new practitioners entering the profession consider it to be the 'Achilles heel' to their repertoire of ET skills, and many seasoned professionals still encounter a difficult transfer in some cows. The ultimate message is that although the ET procedure may appear straightforward, consistent success requires substantial experience and continual refinement of technique. Optimizing outcomes depends not only on advances in embryo production, quality and selection but also on rigorous recipient management and, critically, precise and efficient execution of the transfer procedure.

Authors' contribution statement and agreement

RN: conceptualization and preparation of original manuscript, image collection and preparation, manuscript editing and revision and **CW:** manuscript conceptualization and review. Authors have read and approved final submission.

Conflict of interest

None to report.

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