Pressing needs and recent advances to enhance production of embryos in vitro in cattle

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Abstract

Embryo transfer in cattle is an increasingly important technique for cattle production. Full attainment of the benefits of the technology will depend on overcoming hurdles to optimal performance using embryos produced in vitro. Given its importance, embryo technology research should become a global research priority for animal reproduction science. Among the goals of that research should be developing methods to increase the proportion of oocytes becoming embryos through optimization of in vitro oocyte maturation and in vitro fertilization, producing an embryo competent to establish and maintain pregnancy after transfer, and increasing recipient fertility through selection, management and pharmacological manipulation. The embryo produced in vitro is susceptible to epigenetic reprogramming and methods should be found to minimize deleterious epigenetic change while altering the developmental program of the resultant calf to increase its health and productivity. There are widening opportunities to rethink the technological basis for much of the current practices for production and transfer of embryos because of explosive advances in fields of bioengineering such as microfluidics, three-dimensional printing of cell culture materials, organoid culture, live-cell imaging, and cryopreservation.

Keywords: embryo, blastocyst, in vitro production, cattle, recipient, fertility.

A call to prioritize embryo technology research

Embryo transfer in cattle has been a reality since December 19, 1950 when Elwyn Willet and colleagues at the American Foundation for the Study of Genetics and the University of Wisconsin produced the first calf (named "Prima") derived from transfer of an embryo (Willett et al., 1951; Betteridge, 2000). However, the utility of embryo transfer as a tool for genetic improvement was limited until the introduction of the first high density SNP chip for cattle in 2009 (Matukumalli et al., 2009). This chip made it possible to identify genetically-superior females with high reliability. Until then, genetic progress from embryo transfer was hampered by the fact that accuracy of selecting females was poor when compared to the high degree of accuracy conferred by progeny testing of bulls. The utilization of embryo transfer has almost doubled since the introduction of genotyping platforms. The Data Retrieval Committee of the International Embryo Transfer Association reported 794,397 embryos had been transferred in 2008 (Thibier, 2009). The same committee found that the number of transfers reported in 2022 was 1,558,482 (Viana, 2023).

The importance of embryo technologies will continue to rise. Embryo transfer can increase the rate of genetic selection by increasing the intensity of genetic selection on the female side (VanRaden, 2020) and by shortening the generation interval. Genetic selection can be made as

early as the blastocyst stage (Agerholm et al., 2023). Somatic cell nuclear cloning and production of gene-edited animals are both technologies that depend on production of embryos in the laboratory and which can contribute to genetic improvement of livestock. A more revolutionary event in the future may be the development of "in vitro breeding" where rounds of genetic selection of embryos produced in vitro are followed by generation of stem cells from those embryos that can be differentiated into sperm cells and oocytes to produce the next generation of embryos for selection (Goszczynski et al., 2023). Embryo transfer can also increase the value of beef calves produced from dairy animals (Crowe et al., 2021) and can be used to improve fertility in heat-stressed and repeat-breeder cows and, eventually, more broadly (Hansen, 2020a). Indeed, the potential impact of embryo technologies on cattle production is so broad that this author has speculated that embryo transfer may eventually rival artificial insemination (AI) as an assisted reproduction technique (Hansen, 2023).

The scientific developments that have brought embryo technologies to their current standing were achieved with less investment in research than for other important topics in cattle reproduction. Research in bovine reproduction since the 1980s and earlier has been dominated by efforts to develop ovulation synchronization strategies and to improve fertility in the high-producing dairy cow. A search in PUBMED identified 4,924 papers related to AI in cattle, 3,873 papers related to dairy cow fertility and 2,745 papers related to embryo transfer in cattle (Figure 1). Research efforts toward ovulation synchronization research and improving dairy cow fertility have been enormously successful. Timed AI programs are now routinely implemented on many dairy farms in countries where pharmaceutical use is widely available. A host of protocols have been developed that enhance fertility as well as allow appointment breeding (Fricke and Wiltbank, 2022). Dairy cow fertility has been improved because of an assemblage of advances including increased use of ovulation synchronization programs, improved transition cow health programs, enhanced feeding regimens, and other practices. Ovulation synchronization has also made AI in beef cattle more practical than formerly and can result in more calves born earlier in the calving season (Baruselli et al., 2018; Monteiro et al., 2023).
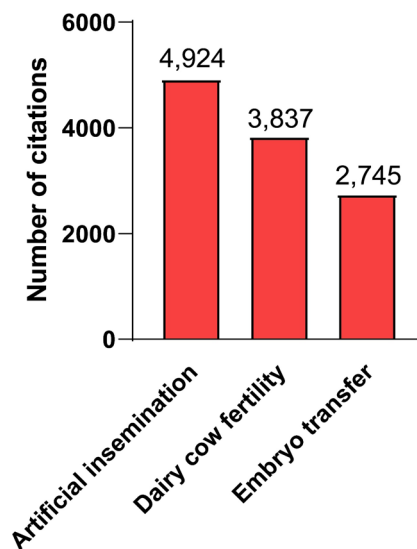

Figure 1. Analysis of PUBMED to assess research activity related to embryo technologies as compared to research focused on artificial insemination and dairy cow fertility. Shown are the number of papers meeting specific search criteria for a search conducted March 7 2024. The search terms related to artificial insemination were "artificial insemination and (cattle or cow or heifer)". The search terms for dairy cow fertility were "(fertility or infertility or fertile or infertile) and cow and (dairy or Holstein or Jersey)". The search terms for embryo transfer were "embryo transfer and (cow or cattle or heifer)".

The potential that embryo technologies present for transforming genetic improvement and improving fertility has been limited by suboptimal processes for producing and transferring embryos. The promise offered by the transferrable embryo, coupled with the current obstacles to optimization, means that research to improve embryo technologies should become national

priorities for those countries in which cattle production is an important economic activity. Now is the time to increase funding for embryo technologies. In this paper, the goal will be to outline particular areas where research should be focused. The topics are not meant to be inclusive and the coverage of the literature is non-exhaustive. The approach is to highlight some areas that are particularly amenable to improvements or where recent advances show promise.

Making a better oocyte and zygote

The production of embryos in vitro is very inefficient. The percent of oocytes that are placed into oocyte maturation medium that later become a transferrable embryo is dependent on the culture system but is usually between 20 and 40%. These values are lower than what can be achieved in vivo. For example, the percent of inseminated heifers that was pregnant at day 7 was 54% in a study with beef animals in Ireland (Carter et al., 2008) and 70.9% for dairy heifers in New Zealand (Berg et al., 2022). A total of 56% of lactating cows inseminated at 40-60 days in milk yielded high- or fair-quality embryos at day 5 or 6 after insemination (Denis-Robichaud et al., 2022).

The low yield of transferrable embryos following procedures for in vitro production is not because oocytes fail to mature or become fertilized. Indeed, rates of nuclear maturation and fertilization are high (>70%). Instead, it is because many fertilized embryos fail to develop adequately in culture. They fail largely because of errors in the process of in vitro maturation and fertilization.

The conclusion that inadequate conditions for maturation and fertilization are a major cause of poor embryo competence are based on experiments in which measures of embryonic development were made for embryos produced in vivo, in vitro or in a combination of both conditions. Results of one such experiment, by Gad et al. (2012), are shown in Figure 2. The percent of oocytes that became blastocysts following in vitro maturation, fertilization and embryonic development was 12.2%. If in vitro produced embryos were transferred to the uterus at the 16-cell stage, there was no improvement in blastocyst development (10.6%) while transfer to the oviduct at the 4-cell stage caused a slight increase in development (26.6%). In contrast, production of embryos by superovulation, followed by flushing from the animal and subsequent culture at either the 4-cell stage or 16-stage resulted in most oocytes becoming blastocysts (83.0% and 69.8%, respectively). What was most crucial to ensuring blastocyst development was the period of oocyte maturation, fertilization, or development through the 4-cell stage. Findings of two earlier experiments by Rizos et al. (2002) also highlighted the importance of oocyte maturation and fertilization. In the first experiment, the percent of putative zygotes (i.e, oocytes exposed to sperm) becoming blastocysts if maturation, fertilization and embryo development occurred in vitro was 39%. The percent rose to 78% if oocyte maturation alone occurred in vivo. In the second experiment, percent blastocyst was 39% for embryos produced totally in vitro, 58% for embryos from oocytes that were matured in vivo but fertilized and allowed to develop in vitro, and 74% for embryos from oocytes where maturation and fertilization but not development occurred in vivo.
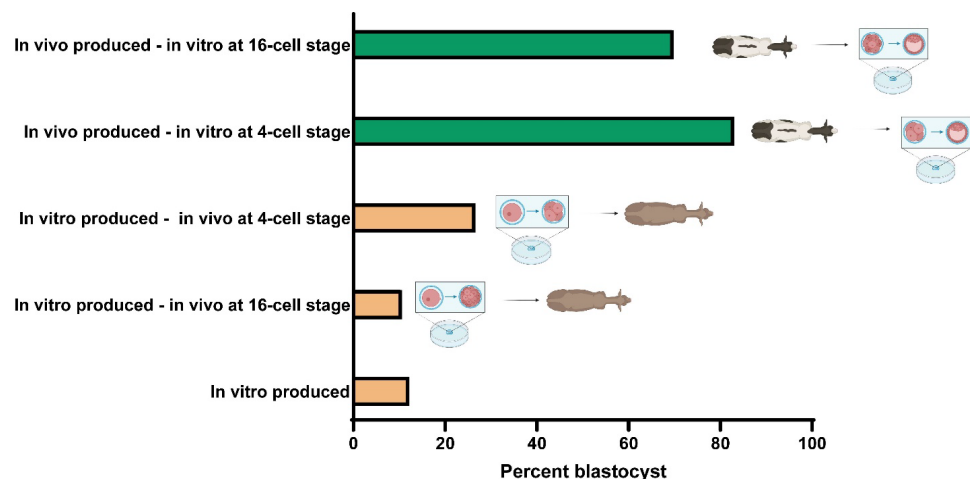

Figure 2. Percent of oocytes becoming blastocysts where embryos were produced in vivo, in vitro or in a combination of both conditions. Data are obtained from Gad et al. (2012).

These results indicate that the zygote formed from in vitro maturation and fertilization is already compromised in its ability to proceed to the blastocyst stage. A similar conclusion can be derived from experiments with other species including the human, mouse and rhesus monkey (Hansen, 2020b). There is a compelling need, therefore, to rethink the conditions for oocyte maturation and fertilization in vitro. Perhaps, the most fruitful approaches will be those that try to mimic in vitro the processes for oocyte maturation and fertilization that occur in vivo. There may also be some prospects to improve blastocyst yield by changing conditions for development after fertilization.

Oocyte maturation

Maturational events in the cumulus-oocyte complex in vivo are driven by luteinizing hormone (LH) and mediators of LH action like amphiregulin, neuroregulin, epiregulin and betacellulin (Strączyńska et al., 2022). Key events include dynamic changes in oocyte concentrations of cGMP and cAMP controlled by loss of gap junctions between cumulus cells and oocytes. Premature loss of oocyte cAMP in vitro is believed to result in premature nuclear maturation and a decoupling of the processes of nuclear and cytoplasmic maturation (Gilchrist and Thompson, 2007). Artificial regulation of oocyte cAMP during in vitro maturation by pharmacological methods has sometimes (but not always) been reported to increase the proportion of oocytes developing to the blastocyst stage (Gilchrist et al., 2016; Leal et al., 2022).

Another approach to improve oocyte maturation in vitro is to add to culture medium specific cell signaling ligands involved in maturation in vivo including follicle stimulating hormone, amphiregulin, insulin-like growth factor 1, estradiol, progesterone, androstenedione, neuroregulin 1 and natriuretic peptide C. Improvements in the characteristics of blastocysts produced have been reported (Soares et al., 2017; Dellaqua et al., 2023) but the percent of oocytes becoming blastocysts is still below what is achieved in vivo. Recently, Zhang et al. (2023) reported that C-X-C motif chemokine ligand 12 acts on oocytes during maturation to increase the percent that become blastocysts following fertilization or parthenogenetic activation.

Fertilization

The experiment by Rizos et al. (2002) comparing blastocyst development for oocytes fertilized in vivo vs in vitro is indicative that conditions for in vitro fertilization are not optimal for developmental competence of the resulting embryo. Sperm cells vary in ability to produce embryos with high developmental competence (Hendricks and Hansen, 2010; Behnam et al., 2023; Vallet-Buisan et al., 2023; Li et al., 2023; Yaghoobi et al., 2024). Perhaps the probability of fertilization with a defective spermatozoan is greater for in vitro fertilization than for fertilization in vivo. Sperm concentration for in vitro fertilization is usually 1×10^6/mL while estimated numbers of sperm in the oviduct after insemination are in the 10's of thousands; the number that reach the site of fertilization are likely to be in the hundreds or lower (Hawk, 1987). It is possible that the sperm winnowing process in the reproductive tract (see Miller, 2024) is such that sperm more fit for fertilization and support of embryonic development have greater likelihood to reach the oocyte than less-fit sperm. In mice, for example, passage through the utero-tubal junction depends upon presence of expression of specific genes in the male such as *Adam3* and *Lypd4* (Fujihara et al., 2019) and sperm with fragmented DNA are less likely to transit the utero-tubal junction (Hourcade et al., 2010).

Given the variability in ability of sperm to support embryonic development, one strategy for improving outcomes of in vitro fertilization is to devise new methods for selecting sperm for fertilization. Selection of sperm based on rheotaxis, for example, increased the proportion of cleaved embryos becoming a blastocyst as compared to oocytes from sperm isolated by centrifugation (Yaghoobi et al., 2024). Another strategy is to mimic sperm-oviductal interactions that occur in vivo. Sperm binding to the oviductal epithelium promotes sperm survival (Pollard et al., 1991) and, based on experiments in rabbits and pigs, the oviductal isthmus is important for reducing polyspermy (Mahé et al., 2021). Capacitation of sperm in vivo involves complex changes in the sperm mediated by the oviduct (Mahé et al., 2021; Delgado-Bermúdez et al., 2022). Capacitation of sperm in vitro is caused by heparin (Parrish, 2014) and it may be that this glycosaminoglycan does not completely mimic oviduct-induced changes in sperm function associated with capacitation. Delayed or incomplete capacitation could conceivably result in fertilization with a defective sperm or oocyte aging, which can reduce embryo competence for development (Koyama et al., 2014). Co-culture of

sperm, oviductal epithelial cells and oocytes in a system termed "oviduct-on-a-chip" resulted in reduced incidence of polyspermy and parthenogenesis (Ferraz et al., 2017).

Embryo culture

Expression of large number of genes encoding for receptors for cell-signaling ligands by the early embryo (Sang et al., 2021; Hoorn et al., 2023) is evidence that the preimplantation embryo is in active communication with the mother. Addition of oviductal fluid (Lopera-Vasquez et al., 2017) or several specific growth factors whose gene is expressed in the oviduct or endometrium can increase the percent of in vitro produced embryos developing to the blastocyst stage. Examples of molecules that can increase blastocyst yield include activin A (Trigal et al., 2011; Kannampuzha-Francis et al., 2017; Tríbulo et al., 2018), colony stimulating factor 2 (CSF2; Dobbs et al., 2013), C-X-C motif chemokine ligand 12 (Zhang et al., 2023), hepatoma-derived growth factor (Gómez et al., 2017), insulin-like growth factor 1 (Tríbulo et al., 2018), WNT5A (Jeensuk et al., 2022), and WNT7A (Tríbulo et al., 2018).

Cell-signaling ligands that can enhance embryonic function have been termed embryokines (Hansen et al., 2014). One of the features of actions of embryokines in vitro is that the magnitude of the increase in blastocyst yield is modest. It is likely that these molecules cannot overcome the reduction in developmental competence caused by inadequate conditions for oocyte maturation or fertilization. In addition, other components of culture medium, most notably albumin, can alter growth factor biological activity (Gómez et al., 2017; Jeensuk et al., 2022). It is also possible that embryos themselves secrete specific embryokines so addition of an exogenous source of the molecule might not be impactful. Recently, it was surmised that the reason why bovine embryos are more likely to become blastocysts when cultured in groups than when cultured singly (Donnay et al., 1997) is because of embryonic secretion of L-cathepsin. This proteinase is secreted in higher amounts by embryos classified as excellent or good than for embryos classified as poor (Raes et al., 2023). Moreover, addition of L-cathepsin to embryos cultured individually increased the proportion that became blastocysts (Raes et al., 2023).

Making a better transferrable embryo

The blastocyst produced in vitro differs from its in vivo produced counterpart in many respects including in terms of ultrastructure, lipid content, gene expression, epigenetic modifications, cell numbers, and incidence of chromosomal abnormalities (Hansen, 2023). Not surprisingly, pregnancy rates achieved following transfer of an in vitro produced embryo are often lower than those achieved following transfer of an embryo produced in vivo by superovulation (Ealy et al., 2019). An example of results from one such study comparing pregnancy outcomes for both kind of embryos, that of Pérez-Mora et al. (2020), is shown in Figure 3. In some reports, but not all, pregnancy losses after the initial pregnancy diagnosis were also greater for pregnancies involving embryos produced in vitro than pregnancies established by AI (reviewed by Hansen, 2023; also see Crowe et al., 2024).
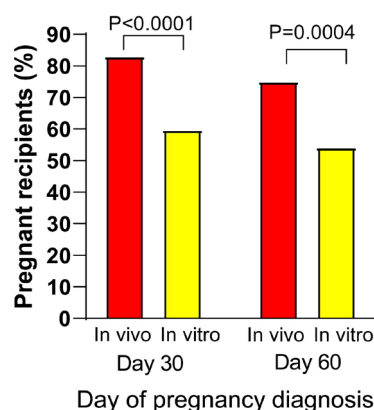

Figure 3. Comparison of pregnancy outcomes for transfer of embryos produced by superovulation (in vivo) or in vitro for beef cows in tropical conditions in Mexico (Pérez-Mora et al., 2020).

It is not known whether reduced competence of the embryo produced in vitro to establish and maintain pregnancy is related to errors associated with oocyte maturation, fertilization, embryo culture or some combination. There are a multitude of experiments describing specific conditions of embryo culture that change blastocyst gene expression, relative or absolute numbers of cells in the trophectoderm (TE) and inner cell mass (ICM), or other characteristics. For example, many of the cell-signaling molecules produced by the endometrium that have embryokine activity can modify allocation of cells of the blastocyst into TE, ICM and hypoblast. Examples of embryokines that have been reported to alter the number of ICM cells in the blastocyst include connective tissue growth factor (Kannampuzha-Francis et al., 2017), C-natriuretic peptide (Sang et al., 2020), interleukin 6 (IL6, Wooldridge et al., 2019; Wooldridge and Ealy, 2021; Seekford et al., 2021), and WNT5A (Jeensuk et al., 2022). Furthermore, Dickopf WNT signaling inhibitor 1 (DKK1), (Denicol et al., 2014), fibroblast growth factor 2 (Yang et al., 2011), and IL6 (Wooldridge and Ealy, 2021) can promote differentiation of hypoblast cells from ICM. Also, DKK1 can increase number of TE cells (Denicol et al., 2014; Amaral et al., 2022a). It is unclear, however, whether any of these embryokines can improve competence of embryos to establish pregnancy. Early experiments indicated that treatment of embryos with CSF2 from day 5 to 7 of development increased pregnancy success after transfer to recipients (Loureiro et al., 2009; Denicol et al., 2014) but a recent meta-analysis of all embryo transfer experiments with CSF2 failed to support the idea that CSF2 increases embryo survival (Hansen et al., 2024). Embryo transfer experiments with DKK1 have also yielded mixed results (Denicol et al., 2014; Amaral et al., 2022b). Pregnancy rates at day 70 for cows receiving an embryo treated with IL6 were numerically but non-significantly higher than for cows receiving control embryos (12/28 vs 11/43) and fetal size for IL6 embryos was more similar to that of fetuses derived by AI than for fetal size of control embryos (Seekford et al., 2021).

One difficulty in understanding determinants of embryo competence for pregnancy is that most embryo transfer experiments designed to test improvements in embryo survival are underpowered. Adequately powered experiments in which pregnancy outcome is the endpoint requires hundreds of observations per treatment. This size of experiment requires resources beyond the reach of most academic laboratories. Commercial laboratories can do these kinds of experiments but it is often not practical to modify a functioning system of embryo production for experimental purposes.

One alternative approach is to identify markers of embryo competence that can be used to rapidly screen potential treatments for improving embryo competence for pregnancy establishment. The most promising treatments could then be tested for efficacy in a large-scale embryo transfer experiment. Recently, Rabaglino et al. (2023) has used machine learning to analyze a variety of datasets on gene expression in bovine embryos including those from demi-blastocysts in which one half of the blastocyst was transferred and one-half was subjected to RNA sequencing. A total of eight genes were identified whose expression together predicted with high accuracy whether pregnancy would result after transfer. Screening of the transcript abundance of these genes could provide insights into the conditions for producing an embryo competent to establish pregnancy. There are also likely to be metabolic signatures of an embryo competent to establish pregnancy as indicated by analysis of spent culture medium of blastocysts that did or did not establish pregnancy after transfer (Oliveira Fernandes et al., 2023).

Another potential method for distinguishing between embryos that are competent or non-competent to establish pregnancy is the use of morphokinetic analysis of embryonic development. Incubators with built-in microscopes and cameras are now available that allow time-lapse imaging of individual embryos as they advance in development (Magata, 2023). Timing of blastocyst formation and specific morphological characteristics of the blastocyst have been found predictive of ability of the embryo to remain viable in culture after day 7.5 (Huayhua et al., 2023). Future experiments to identify morphokinetic determinants of embryonic survival after transfer to recipients could result in a useful tool for increasing embryo competence for pregnancy establishment.

One cause of pregnancy failure in embryos produced in vitro are chromosomal abnormalities. The proportion of embryos with errors in chromosomal segregation is higher for those produced in vitro than for those produced in vivo (Viuff et al., 1999; Tšuiko et al.,

2017). Some of these embryos are probably discarded before transfer – development is slowest for haploid and polypoid embryos, intermediate for aneuploid embryos and fastest in embryos classified as diploid or mixoploid (Kawarsky et al., 1996). Incidence of chromosomal abnormalities is also inversely related to blastocyst quality grade and stage of development (Tutt et al., 2021). Recently, it was demonstrated that embryo biopsies used to estimate genetic merit of embryos can also be analyzed to identify chromosomal abnormalities (Bouwman and Mullaart, 2023). Thus, embryos can be simultaneously screened for both genetic merit and chromosomal abnormalities.

Making a better calf epigenetically

The preimplantation embryo undergoes extensive epigenetic modifications involving removal of epigenetic marks inherited from the egg and sperm followed by acquisition of epigenetic modifications required for differentiation (Zhu et al., 2021). This process can be modified by culture of the embryo (Niemann et al., 2010; Urrego et al., 2017; Canovas et al., 2021; Ming et al., 2021). Differences in DNA methylation between calves produced from embryos produced in vitro vs in vivo also exist at birth (Rabaglino et al., 2022). Some of these neonatal differences could represent conserved changes in the epigenome that occurred in the embryo and others could represent changes in methylation downstream from cellular changes in the blastocyst. At least some changes in DNA methylation occurring in the embryo are not retained in the adult (Vargas et al., 2023).

In any case, there is some evidence that in vitro production can result in changes in function of the animal at maturity. In one study, it was reported that cows derived from in vitro fertilization with reverse-sorted semen had reduced milk production compared to cows derived from embryos produced by AI, superovulation, or in vitro fertilization with conventional semen (Siqueira et al., 2017). In that same study, calf mortality was highest for the reverse sex-sorted group. In contrast, there was no difference in milk yield between cows produced by AI, superovulation or in vitro production (Lafontaine et al., 2023). There was, however, a slight increase in interval from first service to conception in the cows derived by in vitro production. It should be noted that characteristics of the epigenome of the blastocyst produced in vitro depend upon conditions of culture (Canovas et al., 2021; Clare et al., 2021; Tutt et al., 2023). Thus, consequences of in vitro production of embryos could depend on the specific methodology utilized.

The developmental program of the preimplantation embryo is malleable and a variety of changes in the environment of the mother or embryo can program development to affect postnatal phenotype (Hansen et al., 2016; Hansen, 2020b). As a result, it might be possible to alter culture conditions of the preimplantation embryo to enhance postnatal phenotype. Two embryokines and one nutrient have been reported to do so. As shown in Figure 4, CSF2 has been reported to increase postnatal growth in Holstein heifers (Kannampuzha-Francis et al., 2015). No such effect was seen in Brahman calves (Estrada-Cortés et al., 2021a), which unlike Holsteins, were suckled by their dams. In the latter study, though, CSF2 treatment affected deposition of subcutaneous fat. Calves derived from DKK1-treated embryos were larger at birth than calves derived from control embryos but grew slower than controls thereafter (Amaral et al., 2022a). Culture of in vitro produced embryos with the micronutrient choline has been reported to change DNA methylation in the Brahman calf and increase weaning and slaughter weight (Estrada-Cortés et al., 2021b; Haimon et al., 2024).

The most deleterious example of disordered development associated with in vitro production is large offspring syndrome (LOS) or, as it is more accurately termed, abnormal offspring syndrome (Farin et al., 2010). The most obvious representation of the syndrome is the extremely large size at birth of affected calves (Figure 5). Other developmental defects including umbilical hernia, organomegaly, abdominal wall defects, and changes in DNA methylation are associated with the condition (Nava-Trujillo and Rivera, 2023). Once thought to be exclusively associated with in vitro production of embryos and somatic cell nuclear transfer, it is now clear that LOS can occur with natural mating or AI (Nava-Trujillo and Rivera, 2023). There are no reliable estimates on the incidence of the syndrome - even the definition of LOS varies among investigators. In our laboratory, the frequency of calved produced by in

vitro fertilization that have LOS (defined as very large calves that are born dead or die soon after birth) has been about 5%. The fact that the frequency is so low means it will be difficult to perform experiments to identify culture conditions that minimize the incidence. The best solution will be to identify markers of the syndrome in the embryo or pregnant cow and terminate pregnancies with a high probability of development of a LOS calf. Efforts to identify such markers are underway (Rivera et al., 2022).
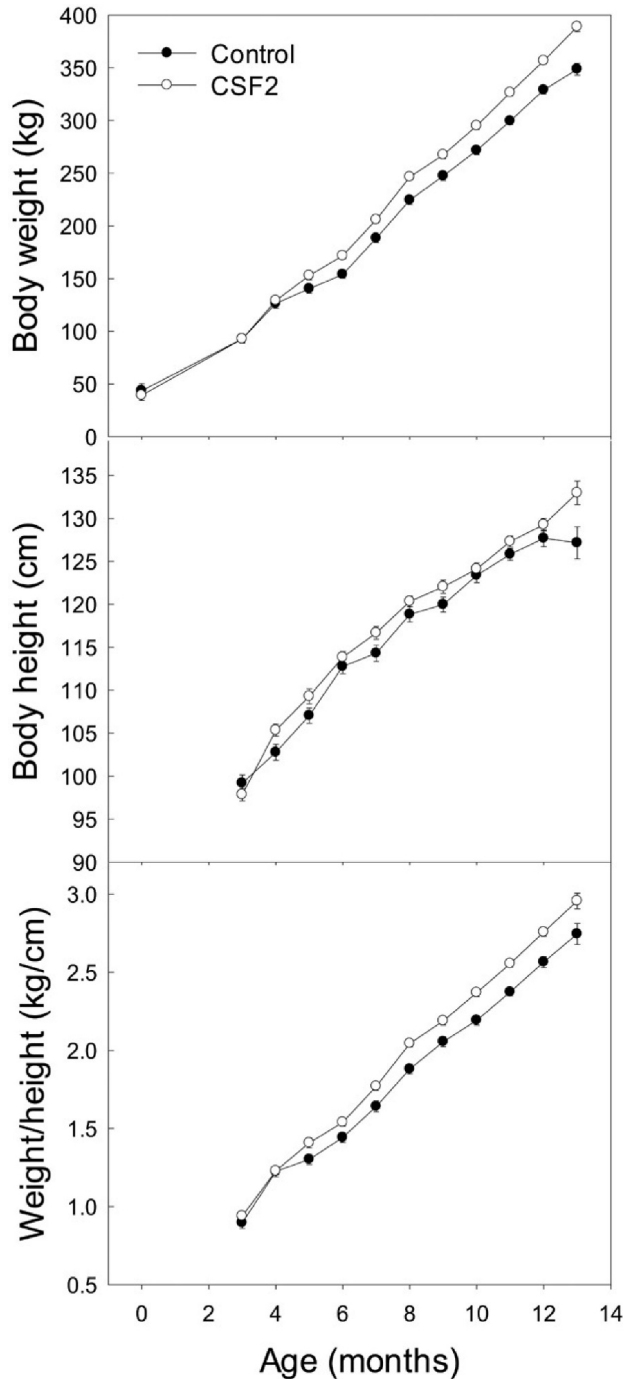

Figure 4. Programming of postnatal growth by exposure of preimplantation embryos to colony stimulating factor 2 (CSF2) from day 5 to 7 of culture. Data are body weights of calves derived from control embryos or embryos cultured with CSF2. Data are least-squares means ± standard error (error bars are not visible when errors were smaller than the symbol). The interaction between treatment and month of age affected weight ($P < 0.001$) and weight-to-height ratio ($P = 0.0089$). The figure is from Kannampuzha-Francis et al. (2015) and is reproduced with permission from *Molecular Reproduction and Development*.

Figure 5. Example of a stillborn bull calf with large offspring syndrome. Body weight was 59 kg and macroglossia was present. The figure is from Amaral et al. (2022a) and is reproduced with permission from *Biology of Reproduction*.

Increasing receptivity of the recipient

Pregnancy success after embryo transfer depends not only on the competence of the embryo to establish pregnancy but also on the ability of the recipient to support pregnancy. Using data from experiments in which two potential pregnancies per female were established, McMillan (1998) estimated that only about 50% of females were capable to maintaining a pregnancy vs 70% of embryos produced in vivo and 60% of embryos produced in vitro. Moreover, experiments in which females underwent repeated rounds of embryo transfer make it clear that some cows are inherently and repeatedly fertile while others are inherently subfertile (Geary et al., 2016). It is to be expected that pregnancy success after embryo transfer will be increased if methods are developed to 1) either identify inherently-fertile cows or 2) to manage cows so that the proportion of recipients that are fertile (i.e., receptive) is increased.

There are physiological characteristics that distinguish cows on their basis for supporting pregnancy. Cows that display estrus in fixed-time embryo transfer procedures have higher pregnancy rates than cows that do not (Cedeño et al., 2020). Blood flow to the corpus luteum at the time of transfer is also positively related to pregnancy outcomes (Kanazawa et al., 2016; Pugliesi et al., 2019; Santos et al., 2023). Other markers of receptivity include expression of specific mRNA (Ponsuksili et al., 2012) and miRNA in the endometrium (Ponsuksili et al., 2014).

Fixed-time embryo transfer is widely used as a management tool for recipients; work continues on optimizing the hormonal treatments employed for this procedure (Pereira et al., 2013; Bonacker et al., 2020). Efforts to improve pregnancy outcomes by modifying the endocrine environment around the time of embryo transfer has yielded mixed results, with reports of both positive effects and lack of effectiveness. This has been the case for treatment with progesterone, GnRH and human chorionic gonadotropin (Block et al., 2003; Monteiro et al., 2015; Niles et al., 2019; García-Guerra et al., 2020; El-Azzi et al., 2023; Chen et al., 2023; Laurindo et al., 2024). A treatment with a more consistent benefit on pregnancy outcomes is administration of flunixin meglumine or other anti-inflammatories at the time of embryo transfer (Besbaci et al., 2021; Barnes et al., 2023). These treatments reduce inflammation associated with the process of transfer itself since they are particularly effective at increasing pregnancy rate in cows in which it was difficult to pass the embryo transfer pipette through the cervix (Besbaci et al., 2021).

Improving embryo freezability

In 2022, 44% of the global total of recorded transfers of embryos produced in vitro involved a cryopreserved embryo (Viana, 2023) even though pregnancy success after transfer of a

cryopreserved embryo remains lower than for transfer of a fresh embryo (Hansen 2020a, 2023). Enhancements in the conditions for production of embryos could conceivably reduce the difference in pregnancy rates between cryopreserved and fresh embryos. So too could enhancements in techniques for cryopreservation. The ideal cryopreservation system would be one where an embryo could be transferred directly without the need for washing and repackaging because transfers could be performed in locations where trained technicians were not available. Gómez et al. (2020) has reported that freezing in an ethylene-glycol based cryoprotectant solution with polyvinyl alcohol instead of bovine serum albumin yielded pregnancy rates similar to those with transfer of fresh embryos [40/80 (50% vs 30/58 (52%). Similarly, Oliveira et al. (2020) reported an in-straw warming protocol for direct transfer of vitrified embryos involving transfer of an embryo from an open vitrification device into an embryo transfer straw held upright and containing liquid columns of 0.15 M sucrose and phosphate-buffered saline. Ten of 25 recipients (40%) receiving such warmed embryos became pregnant as compared to a rate of 43% for recipients receiving fresh embryos. Other experiments have been performed to determine whether addition of various biologically-active molecules to embryos around the time of cryopreservation improves embryonic survival. Examples in which some improvement in survival was reported include antifreeze glycoprotein 8 (Liang et al., 2017), ascorbate (Carrascal-Triana et al., 2022), and an inhibitor of Rho-associated coiled-coil containing kinase (Abdelhady et al., 2023). All of these approaches for enhancing outcomes of embryo cryopreservation are promising but it will be important to perform large-scale embryo transfer experiments to confirm efficacy.

Designing better tools for embryo production and transfer

There are widening opportunities to rethink the technological basis for much of the current practices for production and transfer of embryos. There have been explosive advances in fields of bioengineering such as microfluidics, three-dimensional printing of cell culture materials, organoid culture, live-cell imaging and cryopreservation. Moreover, artificial intelligence will certainly have a role in embryo technologies. Examples of emerging technologies include microfluidics (Suh et al., 2006; Ferraz and Ferronato, 2023; Alkan et al., 2023), artificial tissues (Gargus et al., 2020), three-dimensional culture (Miles et al., 2017; Ferraz and Ferronato, 2023), non-invasive assessment of cellular function (Sciorio et al., 2022), time-lapse imaging (Magata, 2023) and new advances in cryopreservation (Pomeroy et al., 2022). Even a technique as central to the field as transcervical embryo transfer could be re-engineered to avoid possible damage to the reproductive tract (see Hansen, 2020a for discussion). Could not an automated, autonomous device be designed that could traverse the cervix with minimal physical damage to tissues and without the need for technicians with specialized training?

Acknowledgements

References

Abdelhady AWA, Aguiar LH, Lee YL, Guo Z, Bovell RT, Crane PL, Diel de Amorim M, Cheong SH. Rho-associated coiled-coil containing kinase inhibitor improves outcomes of direct-transfer slow-cooled bovine blastocysts. Theriogenology. 2023;211:19-27. http://doi.org/10.1016/j.theriogenology.2023.07.030. PMid:37556931.

Agerholm JS, Madsen SE, Krogh AKH, Najafzadeh V, Secher JB. Health assessment of Holstein calves born after in vitro fertilization, biopsy-based genotyping at the blastocyst stage and subsequent embryo transfer. Theriogenology. 2023;211:76-83. http://doi.org/10.1016/j.theriogenology.2023.08.005. PMid:37595376.

Alkan H, Satilmis F, Demirel MA, Bodu M, Yesilkaya OF, Ciftci MF, Erdem H, Tekindal MA, Alkan KK. Does using microfluidic sperm sorting chips in bovine IVEP affect blastocyst development? Reprod Domest Anim. 2023;58(7):1012-20. http://doi.org/10.1111/rda.14398. PMid:37246427.

Amaral TF, Gonella-Diaza A, Heredia D, Melo GD, Estrada-Cortés E, Jensen LM, Pohler K, Hansen PJ. Actions of DKK1 on the preimplantation bovine embryo to affect pregnancy establishment, placental function, and postnatal phenotype. Biol Reprod. 2022a;107(4):945-55. http://doi.org/10.1093/biolre/ioac128. PMid:35765194.

Amaral TF, de Grazia JGV, Martinhao LAG, De Col F, Siqueira LGB, Viana JHM, Hansen PJ. Actions of CSF2 and DKK1 on bovine embryo development and pregnancy outcomes are affected by composition of embryo culture medium. Sci Rep. 2022b;12(1):7503. http://doi.org/10.1038/s41598-022-11447-7. PMid:35525843.

Barnes M, Kasimanickam R, Kasimanickam V. Effect of subclinical endometritis and flunixin meglumine administration on pregnancy in embryo recipient beef cows. Theriogenology. 2023;201:76-82. http://doi.org/10.1016/j.theriogenology.2023.02.020. PMid:36842264.

Baruselli PS, Ferreira RM, Sá MF Fo, Bó GA. Review: using artificial insemination v. natural service in beef herds. Animal. 2018;12(s1):s45-52. http://doi.org/10.1017/S175173111800054X. PMid:29554986.

Behnam M, Asadpour R, Topraggaleh TR, Hamali H. Improvement of post-thaw quality and fertilizing ability of bull spermatozoa using Rho kinase inhibitor in freezing extender. Front Vet Sci. 2023;10:1155048. http://doi.org/10.3389/fvets.2023.1155048. PMid:37483290.

Berg DK, Ledgard A, Donnison M, McDonald R, Henderson HV, Meier S, Juengel JL, Burke CR. The first week following insemination is the period of major pregnancy failure in pasture-grazed dairy cows. J Dairy Sci. 2022;105(11):9253-70. http://doi.org/10.3168/jds.2021-21773. PMid:36153157.

Besbaci M, Abdelli A, Belabdi I, Raboisson D. Non-steroidal anti-inflammatory drugs at embryo transfer on pregnancy rates in cows: a meta-analysis. Theriogenology. 2021;171:64-71. http://doi.org/10.1016/j.theriogenology.2021.04.010. PMid:34029785.

Betteridge KJ. Reflections on the golden anniversary of the first embryo transfer to produce a calf. Theriogenology. 2000;53(1):3-10. http://doi.org/10.1016/S0093-691X(99)00235-6. PMid:10735057.

Block J, Drost M, Monson RL, Rutledge JJ, Rivera RM, Paula-Lopes FF, Ocon OM, Krininger CE 3rd, Liu J, Hansen PJ. Use of insulin-like growth factor-I during embryo culture and treatment of recipients with gonadotropin-releasing hormone to increase pregnancy rates following the transfer of in vitro-produced embryos to heat-stressed, lactating cows. J Anim Sci. 2003;81(6):1590-602. http://doi.org/10.2527/2003.8161590x. PMid:12817508.

Bonacker RC, Gray KR, Breiner CA, Anderson JM, Patterson DJ, Spinka CM, Thomas JM. Comparison of the 7 & 7 Synch protocol and the 7-day CO-Synch + CIDR protocol among recipient beef cows in an embryo transfer program. Theriogenology. 2020;158:490-6. http://doi.org/10.1016/j.theriogenology.2020.09.033. PMid:33080452.

Bouwman AC, Mullaart E. Screening of in vitro-produced cattle embryos to assess incidence and characteristics of unbalanced chromosomal aberrations. JDS Commun. 2023;4(2):101-5. http://doi.org/10.3168/jdsc.2022-0275. PMid:36974223.

Canovas S, Ivanova E, Hamdi M, Perez-Sanz F, Rizos D, Kelsey G, Coy P. Culture medium and sex drive epigenetic reprogramming in preimplantation bovine embryos. Int J Mol Sci. 2021;22(12):6426. http://doi.org/10.3390/ijms22126426. PMid:34204008.

Carrascal-Triana EL, Zolini AM, de King AR, Penitente-Filho JM, Hansen PJ, Torres CAA, Block J. Effect of addition of ascorbate, dithiothreitol or a caspase-3 inhibitor to cryopreservation medium on post-thaw survival of bovine embryos produced in vitro. Reprod Domest Anim. 2022;57(9):1074-81. http://doi.org/10.1111/rda.14182. PMid:35699342.

Carter F, Forde N, Duffy P, Wade M, Fair T, Crowe MA, Evans AC, Kenny DA, Roche JF, Lonergan P. Effect of increasing progesterone concentration from day 3 of pregnancy on subsequent embryo survival and development in beef heifers. Reprod Fertil Dev. 2008;20(3):368-75. http://doi.org/10.1071/RD07204. PMid:18402756.

Cedeño A, Tríbulo A, Tríbulo RJ, Andrada S, Mapletoft RJ, Bó GA. Effect of estrus expression or treatment with GnRH on pregnancies per embryo transfer and pregnancy losses in beef recipients synchronized with estradiol/progesterone-based protocols. Theriogenology. 2020;157:378-87. http://doi.org/10.1016/j.theriogenology.2020.08.023. PMid:32866844.

Chen F, Hou Y, Zhu X, Mei C, Guo R, Shi Z. Impact of accessory corpus luteum induced by gonadotropin-releasing hormone or human chorionic gonadotropin on pregnancy rates of dairy cattle following embryo transfer: A META-analysis. Vet Sci. 2023;10(5):309. http://doi.org/10.3390/vetsci10050309. PMid:37235391.

Clare CE, Pestinger V, Kwong WY, Tutt DAR, Xu J, Byrne HM, Barrett DA, Emes RD, Sinclair KD. Interspecific variation in one-carbon metabolism within the ovarian follicle, oocyte, and preimplantation embryo: consequences for epigenetic programming of DNA methylation. Int J Mol Sci. 2021;22(4):1838. http://doi.org/10.3390/ijms22041838. PMid:33673278.

Crowe AD, Lonergan P, Butler ST. Invited review: use of assisted reproduction techniques to accelerate genetic gain and increase value of beef production in dairy herds. J Dairy Sci. 2021;104(12):12189-206. http://doi.org/10.3168/jds.2021-20281. PMid:34538485.

Crowe AD, Sánchez JM, Moore SG, McDonald M, Rodrigues R, Morales MF, Orsi de Freitas L, Randi F, Furlong J, Browne JA, Rabaglino MB, Lonergan P, Butler ST. Fertility in seasonal-calving pasture-based lactating dairy cows following timed artificial insemination or timed embryo transfer with fresh or frozen in vitro-produced embryos. J Dairy Sci. 2024;107(3):1788-804. http://doi.org/10.3168/jds.2023-23520. PMid:37806631.

Delgado-Bermúdez A, Yeste M, Bonet S, Pinart E. A review on the role of bicarbonate and proton transporters during sperm capacitation in mammals. Int J Mol Sci. 2022;23(11):6333. http://doi.org/10.3390/ijms23116333. PMid:35683013.

Dellaqua TT, Vígaro RA, Janini LCZ, Dal Canto M, Renzini MM, Lodde V, Luciano AM, Buratini J. Neuregulin 1 (NRG1) modulates oocyte nuclear maturation during IVM and improves post-IVF embryo development. Theriogenology. 2023;195:209-16. http://doi.org/10.1016/j.theriogenology.2022.10.041. PMid:36368115.

Denicol AC, Block J, Kelley DE, Pohler KG, Dobbs KB, Mortensen CJ, Ortega MS, Hansen PJ. The WNT signaling antagonist Dickkopf-1 directs lineage commitment and promotes survival of the preimplantation embryo. FASEB J. 2014;28(9):3975-86. http://doi.org/10.1096/fj.14-253112. PMid:24858280.

Denis-Robichaud J, Fernandes ACC, Santos JEP, Cerri RLA. Circulating progesterone at insemination and accessory spermatozoa are associated with fertilization and embryo quality five or six days post insemination in dairy cattle. Theriogenology. 2022;189:64-9. http://doi.org/10.1016/j.theriogenology.2022.04.018. PMid:35724454.

Dobbs KB, Khan FA, Sakatani M, Moss JI, Ozawa M, Ealy AD, Hansen PJ. Regulation of pluripotency of inner cell mass and growth and differentiation of trophectoderm of the bovine embryo by colony stimulating factor 2. Biol Reprod. 2013;89(6):141. http://doi.org/10.1095/biolreprod.113.113183. PMid:24198123.

Donnay I, van Langendonckt A, Auquier P, Grisart B, Vansteenbrugge A, Massip A, Dessy F. Effects of co-culture and embryo number on the in vitro development of bovine embryos. Theriogenology. 1997;47(8):1549-61. http://doi.org/10.1016/S0093-691X(97)00160-X. PMid:16728097.

Ealy AD, Wooldridge LK, McCoski SR. BOARD INVITED REVIEW: post-transfer consequences of in vitro-produced embryos in cattle. J Anim Sci. 2019;97(6):2555-68. http://doi.org/10.1093/jas/skz116. PMid:30968113.

El Azzi MS, Cardoso JL, Landeo RA, Pontes JHF, de Souza JC, Martins JPN. Effect of inducing accessory corpus luteum formation with gonadotropin-releasing hormone or human chorionic gonadotropin on the day of embryo transfer on fertility of recipient dairy heifers and lactating cows. JDS Commun. 2023;4(2):155-60. http://doi.org/10.3168/jdsc.2022-0286. PMid:36974210.

Estrada-Cortés E, Jannaman EA, Block J, Amaral TF, Hansen PJ. Programming of postnatal phenotype caused by exposure of cultured embryos from Brahman cattle to colony-stimulating factor 2 and serum. J Anim Sci. 2021a;99(8):skab180. http://doi.org/10.1093/jas/skab180. PMid:34079989.

Estrada-Cortés E, Ortiz W, Rabaglino MB, Block J, Rae O, Jannaman EA, Xiao Y, Hansen PJ. Choline acts during preimplantation development of the bovine embryo to program postnatal growth and alter muscle DNA methylation. FASEB J. 2021b;35(10):e21926. http://doi.org/10.1096/fj.202100991R. PMid:34533870.

Farin CE, Farmer WT, Farin PW. Pregnancy recognition and abnormal offspring syndrome in cattle. Reprod Fertil Dev. 2010;22(1):75-87. http://doi.org/10.1071/RD09217. PMid:20003848.

Ferraz MAMM, Ferronato GA. Opportunities involving microfluidics and 3D culture systems to the in vitro embryo production. Anim Reprod. 2023;20(2):e20230058. http://doi.org/10.1590/1984-3143-ar2023-0058. PMid:37638255.

Ferraz MAMM, Henning HHW, Costa PF, Malda J, Melchels FP, Wubbolts R, Stout TAE, Vos PLAM, Gadella BM. Improved bovine embryo production in an oviduct-on-a-chip system: prevention of poly-spermic fertilization and parthenogenic activation. Lab Chip. 2017;17(5):905-16. http://doi.org/10.1039/C6LC01566B. PMid:28194463.

Fricke PM, Wiltbank MC. Symposium review: the implications of spontaneous versus synchronized ovulations on the reproductive performance of lactating dairy cows. J Dairy Sci. 2022;105(5):4679-89. http://doi.org/10.3168/jds.2021-21431. PMid:35307178.

Fujihara Y, Noda T, Kobayashi K, Oji A, Kobayashi S, Matsumura T, Larasati T, Oura S, Kojima-Kita K, Yu Z, Matzuk MM, Ikawa M. Identification of multiple male reproductive tract-specific proteins that regulate sperm migration through the oviduct in mice. Proc Natl Acad Sci USA. 2019;116(37):18498-506. http://doi.org/10.1073/pnas.1908736116. PMid:31455729.

Gad A, Hoelker M, Besenfelder U, Havlicek V, Cinar U, Rings F, Held E, Dufort I, Sirard MA, Schellander K, Tesfaye D. Molecular mechanisms and pathways involved in bovine embryonic genome activation and their regulation by alternative in vivo and in vitro culture conditions. Biol Reprod. 2012;87(4):100. http://doi.org/10.1095/biolreprod.112.099697. PMid:22811576.

García-Guerra A, Sala RV, Carrenho-Sala L, Baez GM, Motta JCL, Fosado M, Moreno JF, Wiltbank MC. Postovulatory treatment with GnRH on day 5 reduces pregnancy loss in recipients receiving an in vitro produced expanded blastocyst. Theriogenology. 2020;141:202-10. http://doi.org/10.1016/j.theriogenology.2019.05.010. PMid:31606718.

Gargus ES, Rogers HB, McKinnon KE, Edmonds ME, Woodruff TK. Engineered reproductive tissues. Nat Biomed Eng. 2020;4(4):381-93. http://doi.org/10.1038/s41551-020-0525-x. PMid:32251392.

Geary TW, Burns GW, Moraes JG, Moss JI, Denicol AC, Dobbs KB, Ortega MS, Hansen PJ, Wehrman ME, Neibergs H, O'Neil E, Behura S, Spencer TE. Identification of beef heifers with superior uterine capacity for pregnancy. Biol Reprod. 2016;95(2):47. http://doi.org/10.1095/biolreprod.116.141390. PMid:27417907.

Gilchrist RB, Luciano AM, Richani D, Zeng HT, Wang X, Vos MD, Sugimura S, Smitz J, Richard FJ, Thompson JG. Oocyte maturation and quality: role of cyclic nucleotides. Reproduction. 2016;152(5):R143-57. http://doi.org/10.1530/REP-15-0606. PMid:27422885.

Gilchrist RB, Thompson JG. Oocyte maturation: emerging concepts and technologies to improve developmental potential in vitro. Theriogenology. 2007;67(1):6-15. http://doi.org/10.1016/j.theriogenology.2006.09.027. PMid:17092551.

Gómez E, Carrocera S, Martín D, Pérez-Jánez JJ, Prendes J, Prendes JM, Vázquez A, Murillo A, Gimeno I, Muñoz M. Efficient one-step direct transfer to recipients of thawed bovine embryos cultured in vitro and frozen in chemically defined medium. Theriogenology. 2020;146:39-47. http://doi.org/10.1016/j.theriogenology.2020.01.056. PMid:32036059.

Gómez E, Carrocera S, Martin D, Sánchez-Calabuig MJ, Gutiérrez-Adán A, Murillo A, Muñoz M. Hepatoma-derived growth factor: protein quantification in uterine fluid, gene expression in endometrial-cell culture and effects on in vitro embryo development, pregnancy and birth. Theriogenology. 2017;96:118-25. http://doi.org/10.1016/j.theriogenology.2017.04.008. PMid:28532827.

Goszczynski DE, Navarro M, Mutto AA, Ross PJ. Review: embryonic stem cells as tools for in vitro gamete production in livestock. Animal. 2023;17(Suppl 1):100828. http://doi.org/10.1016/j.animal.2023.100828. PMid:37567652.

Haimon MLJ, Estrada-Cortés E, Amaral TF, Martin H, Jeensuk S, Block J, Heredia D, Venturini M, Santos Rojas C, Gonella-Diaza AM, DiLorenzo N, Scheffler TL, Dufour P, Sirard MA, Dalmaso de Melo G, Pohler KG, Hansen PJ. Provision of choline chloride to the bovine preimplantation embryo alters postnatal body size and DNA methylation. Biol Reprod. 2024;ioae092. http://doi.org/10.1093/biolre/ioae092.

Hansen PJ, Dobbs KB, Denicol AC, Siqueira LGB. Sex and the preimplantation embryo: implications of sexual dimorphism in the preimplantation period for maternal programming of embryonic development. Cell Tissue Res. 2016;363(1):237-47. http://doi.org/10.1007/s00441-015-2287-4. PMid:26391275.

Hansen PJ, Dobbs KB, Denicol AC. Programming of the preimplantation embryo by the embryokine colony stimulating factor 2. Anim Reprod Sci. 2014;149(1-2):59-66. http://doi.org/10.1016/j.anireprosci.2014.05.017. PMid:24954585.

Hansen PJ. The incompletely fulfilled promise of embryo transfer in cattle-why aren't pregnancy rates greater and what can we do about it? J Anim Sci. 2020a;98(11):skaa288. http://doi.org/10.1093/jas/skaa288. PMid:33141879.

Hansen PJ. Implications of assisted reproductive technologies for pregnancy outcomes in mammals. Annu Rev Anim Biosci. 2020b;8:395-413. http://doi.org/10.1146/annurev-animal-021419-084010. PMid:32069434.

Hansen PJ. Some challenges and unrealized opportunities toward widespread use of the *in vitro*-produced embryo in cattle production. Animal. 2023;17(Suppl 1):100745. http://doi.org/10.1016/j.animal.2023.100745. PMid:37567654.

Hansen PJ, Estrada-Cortés E, Amaral TF, Ramírez-Hernández R. Meta-analysis to determine efficacy of colony-stimulating factor 2 for improving pregnancy success after embryo transfer in cattle. Theriogenology. 2024;219:126-31. http://doi.org/10.1016/j.theriogenology.2024.02.025. PMid:38428334.

Hawk HW. Transport and fate of spermatozoa after insemination of cattle. J Dairy Sci. 1987;70(7):1487-503. http://doi.org/10.3168/jds.S0022-0302(87)80173-X. PMid:3305615.

Hendricks KE, Hansen PJ. Consequences for the bovine embryo of being derived from a spermatozoon subjected to oxidative stress. Aust Vet J. 2010;88(8):307-10. http://doi.org/10.1111/j.1751-0813.2010.00585.x. PMid:20633166.

Hoorn QA, Rabaglino MB, Maia TS, Sagheer M, Fuego D, Jiang Z, Hansen PJ. Transcriptomic profiling of the bovine endosalpinx and endometrium to identify putative embryokines. Physiol Genomics. 2023;55(11):557-64. http://doi.org/10.1152/physiolgenomics.00064.2023. PMid:37720990.

Hourcade JD, Pérez-Crespo M, Fernández-González R, Pintado B, Gutiérrez-Adán A. Selection against spermatozoa with fragmented DNA after postovulatory mating depends on the type of damage. Reprod Biol Endocrinol. 2010;8(1):9. http://doi.org/10.1186/1477-7827-8-9. PMid:20113521.

Huayhua C, Rodríguez M, Vega J, Briones M, Rodriguez-Alvarez L, Mellisho E. Blastulation time measured with time-lapse system can predict in vitro viability of bovine blastocysts. PLoS One. 2023;18(8):e0289751. http://doi.org/10.1371/journal.pone.0289751. PMid:37561791.

Jeensuk S, Ortega MS, Saleem M, Hawryluk B, Scheffler TL, Hansen PJ. Actions of WNT family member 5A to regulate characteristics of development of the bovine preimplantation embryo. Biol Reprod. 2022;107(4):928-44. http://doi.org/10.1093/biolre/ioac127. PMid:35765196.

Kanazawa T, Seki M, Ishiyama K, Kubo T, Kaneda Y, Sakaguchi M, Izaike Y, Takahashi T. Pregnancy prediction on the day of embryo transfer (Day 7) and Day 14 by measuring luteal blood flow in dairy cows. Theriogenology. 2016;86(6):1436-44. http://doi.org/10.1016/j.theriogenology.2016.05.001. PMid:27262885.

Kannampuzha-Francis J, Denicol AC, Loureiro B, Kaniyamattam K, Ortega MS, Hansen PJ. Exposure to colony stimulating factor 2 during preimplantation development increases postnatal growth in cattle. Mol Reprod Dev. 2015;82(11):892-7. http://doi.org/10.1002/mrd.22533. PMid:26227079.

Kannampuzha-Francis J, Tribulo P, Hansen PJ. Actions of activin A, connective tissue growth factor, hepatocyte growth factor and teratocarcinoma-derived growth factor 1 on the development of the bovine preimplantation embryo. Reprod Fertil Dev. 2017;29(7):1329-39. http://doi.org/10.1071/RD16033. PMid:27185102.

Kawarsky SJ, Basrur PK, Stubbings RB, Hansen PJ, King WA. Chromosomal abnormalities in bovine embryos and their influence on development. Biol Reprod. 1996;54(1):53-9. http://doi.org/10.1095/biolreprod54.1.53. PMid:8838000.

Koyama K, Kang SS, Huang W, Yanagawa Y, Takahashi Y, Nagano M. Aging-related changes in in vitro-matured bovine oocytes: oxidative stress, mitochondrial activity and ATP content after nuclear maturation. J Reprod Dev. 2014;60(2):136-42. http://doi.org/10.1262/jrd.2013-115. PMid:24492658.

Lafontaine S, Labrecque R, Blondin P, Cue RI, Sirard MA. Comparison of cattle derived from in vitro fertilization, multiple ovulation embryo transfer, and artificial insemination for milk production and fertility traits. J Dairy Sci. 2023;106(6):4380-96. http://doi.org/10.3168/jds.2022-22736. PMid:37028966.

Laurindo AL No, Ruas FL, Santo BSE, Mattos ACD, Silva JB, Diniz MF Jr, Pereira DFC, Lacerda IP, Carvalho JO, Pugliesi G. Effects of long-acting injectable progesterone supplementation at early dioestrus on pregnancy maintenance in beef and dairy recipient cattle. Reprod Domest Anim. 2024;59(1):e14509. http://doi.org/10.1111/rda.14509. PMid:38037714.

Leal GR, Monteiro CAS, Carvalheira LR, Souza-Fabjan JMG. The Simulated Physiological Oocyte Maturation (SPOM) system in domestic animals: A systematic review. Theriogenology. 2022;188:90-9. http://doi.org/10.1016/j.theriogenology.2022.05.023. PMid:35688043.

Li H, Wang Z, Zhao B, Zhang H, Fan D, Ma H, Zhang Y, Wang Y. Sperm-borne lncRNA loc100847420 improves development of early bovine embryos. Anim Reprod Sci. 2023;257:107333. http://doi.org/10.1016/j.anireprosci.2023.107333. PMid:37729849.

Liang S, Yuan B, Jin YX, Zhang JB, Bang JK, Kim NH. Effects of antifreeze glycoprotein 8 (AFGP8) supplementation during vitrification on the in vitro developmental capacity of expanded bovine blastocysts. Reprod Fertil Dev. 2017;29(11):2140-8. http://doi.org/10.1071/RD16426. PMid:28241901.

Lopera-Vasquez R, Hamdi M, Maillo V, Lloreda V, Coy P, Gutierrez-Adan A, Bermejo-Alvarez P, Rizos D. Effect of bovine oviductal fluid on development and quality of bovine embryos produced in vitro. Reprod Fertil Dev. 2017;29(3):621-9. http://doi.org/10.1071/RD15238. PMid:26462440.

Loureiro B, Bonilla L, Block J, Fear JM, Bonilla AQ, Hansen PJ. Colony-stimulating factor 2 (CSF-2) improves development and posttransfer survival of bovine embryos produced in vitro. Endocrinology. 2009;150(11):5046-54. http://doi.org/10.1210/en.2009-0481. PMid:19797121.

Magata F. Time-lapse monitoring technologies for the selection of bovine in vitro fertilized embryos with high implantation potential. J Reprod Dev. 2023;69(2):57-64. http://doi.org/10.1262/jrd.2022-131. PMid:36775299.

Mahé C, Zlotkowska AM, Reynaud K, Tsikis G, Mermillod P, Druart X, Schoen J, Saint-Dizier M. Sperm migration, selection, survival, and fertilizing ability in the mammalian oviduct†. Biol Reprod. 2021;105(2):317-31. http://doi.org/10.1093/biolre/ioab105. PMid:34057175.

Matukumalli LK, Lawley CT, Schnabel RD, Taylor JF, Allan MF, Heaton MP, O'Connell J, Moore SS, Smith TP, Sonstegard TS, van Tassell CP. Development and characterization of a high density SNP genotyping assay for cattle. PLoS One. 2009;4(4):e5350. http://doi.org/10.1371/journal.pone.0005350. PMid:19390634.

McMillan WH. Statistical models predicting embryo survival to term in cattle after embryo transfer. Theriogenology. 1998;50(7):1053-70. http://doi.org/10.1016/S0093-691X(98)00207-6. PMid:10734423.

Miles JR, Laughlin TD, Sargus-Patino CN, Pannier AK. In vitro porcine blastocyst development in three-dimensional alginate hydrogels. Mol Reprod Dev. 2017;84(9):775-87. http://doi.org/10.1002/mrd.22814. PMid:28407335.

Miller DJ. Sperm in the mammalian female reproductive tract: surfing through the tract to try to beat the odds. Annu Rev Anim Biosci. 2024;12(1):301-19. http://doi.org/10.1146/annurev-animal-021022-040629. PMid:37906840.

Ming H, Sun J, Pasquariello R, Gatenby L, Herrick JR, Yuan Y, Pinto CR, Bondioli KR, Krisher RL, Jiang Z. The landscape of accessible chromatin in bovine oocytes and early embryos. Epigenetics. 2021;16(3):300-12. http://doi.org/10.1080/15592294.2020.1795602. PMid:32663104.

Monteiro PL Jr, Nascimento AB, Pontes GC, Fernandes GO, Melo LF, Wiltbank MC, Sartori R. Progesterone supplementation after ovulation: effects on corpus luteum function and on fertility of dairy cows subjected to AI or ET. Theriogenology. 2015;84(7):1215-24. http://doi.org/10.1016/j.theriogenology.2015.06.023. PMid:26255222.

Monteiro PLJ, Consentini CEC, Andrade JPN, Beard AD, Garcia-Guerra A, Sartori R, Wiltbank MC. Research on timed AI in beef cattle: Past, present and future, a 27-year perspective. Theriogenology. 2023;211:161-71. http://doi.org/10.1016/j.theriogenology.2023.07.037. PMid:37639998.

Nava-Trujillo H, Rivera RM. Review: large offspring syndrome in ruminants: current status and prediction during pregnancy. Animal. 2023;17(Suppl 1):100740. http://doi.org/10.1016/j.animal.2023.100740. PMid:37567678.

Niemann H, Carnwath JW, Herrmann D, Wieczorek G, Lemme E, Lucas-Hahn A, Olek S. DNA methylation patterns reflect epigenetic reprogramming in bovine embryos. Cell Reprogram. 2010;12(1):33-42. http://doi.org/10.1089/cell.2009.0063. PMid:20132011.

Niles AM, Fricke HP, Carvalho PD, Wiltbank MC, Hernandez LL, Fricke PM. Effect of treatment with human chorionic gonadotropin 7 days after artificial insemination or at the time of embryo transfer on reproductive outcomes in nulliparous Holstein heifers. J Dairy Sci. 2019;102(3):2593-606. http://doi.org/10.3168/jds.2018-15588. PMid:30692012.

Oliveira CS, Feuchard VLDS, de Freitas C, Rosa PMDS, Camargo AJDR, Saraiva NZ. In-straw warming protocol improves survival of vitrified embryos and allows direct transfer in cattle. Cryobiology. 2020;97:222-5. http://doi.org/10.1016/j.cryobiol.2020.02.007. PMid:32126213.

Oliveira Fernandes G, de Lima CB, Fidelis AAG, Milazzotto MP, Dode MAN. Metabolic signature of spent culture media shows lipid metabolism as a determinant of pregnancy outcomes. Reprod Domest Anim. 2023;58(1):117-28. http://doi.org/10.1111/rda.14271. PMid:36156318.

Parrish JJ. Bovine in vitro fertilization: in vitro oocyte maturation and sperm capacitation with heparin. Theriogenology. 2014;81(1):67-73. http://doi.org/10.1016/j.theriogenology.2013.08.005. PMid:24274411.

Pereira MH, Sanches CP, Guida TG, Rodrigues AD, Aragon FL, Veras MB, Borges PT, Wiltbank MC, Vasconcelos JL. Timing of prostaglandin F2α treatment in an estrogen-based protocol for timed artificial insemination or timed embryo transfer in lactating dairy cows. J Dairy Sci. 2013;96(5):2837-46. http://doi.org/10.3168/jds.2012-5840. PMid:23498008.

Pérez-Mora A, Segura-Correa JC, Peralta-Torres JA. Factors associated with pregnancy rate in fixed-time embryo transfer in cattle under humid-tropical conditions of México. Anim Reprod. 2020;17(2):e20200007. http://doi.org/10.1590/1984-3143-ar2020-0007. PMid:32714459.

Pollard JW, Plante C, King WA, Hansen PJ, Betteridge KJ, Suarez SS. Fertilizing capacity of bovine sperm may be maintained by binding of oviductal epithelial cells. Biol Reprod. 1991;44(1):102-7. http://doi.org/10.1095/biolreprod44.1.102. PMid:2015341.

Pomeroy KO, Comizzoli P, Rushing JS, Lersten IL, Nel-Themaat L. The ART of cryopreservation and its changing landscape. Fertil Steril. 2022;117(3):469-76. http://doi.org/10.1016/j.fertnstert.2022.01.018. PMid:35219471.

Ponsuksili S, Murani E, Schwerin M, Schellander K, Tesfaye D, Wimmers K. Gene expression and DNA-methylation of bovine pretransfer endometrium depending on its receptivity after in vitro-produced embryo transfer. PLoS One. 2012;7(8):e42402. http://doi.org/10.1371/journal.pone.0042402. PMid:22952593.

Ponsuksili S, Tesfaye D, Schellander K, Hoelker M, Hadlich F, Schwerin M, Wimmers K. Differential expression of miRNAs and their target mRNAs in endometria prior to maternal recognition of pregnancy associates with endometrial receptivity for in vivo- and in vitro-produced bovine embryos. Biol Reprod. 2014;91(6):135. http://doi.org/10.1095/biolreprod.114.121392. PMid:25253731.

Pugliesi G, Dalmaso de Melo G, Silva JB, Carvalhêdo AS, Lopes E, de Siqueira Filho E, Silva LA, Binelli M. Use of color-Doppler ultrasonography for selection of recipients in timed-embryo transfer programs in beef cattle. Theriogenology. 2019;135:73-9. http://doi.org/10.1016/j.theriogenology.2019.06.006. PMid:31203090.

Rabaglino MB, Salilew-Wondim D, Zolini A, Tesfaye D, Hoelker M, Lonergan P, Hansen PJ. Machine-learning methods applied to integrated transcriptomic data from bovine blastocysts and elongating conceptuses to identify genes predictive of embryonic competence. FASEB J. 2023;37(3):e22809. http://doi.org/10.1096/fj.202201977R. PMid:36753406.

Rabaglino MB, Secher JB, Hyttel P, Kadarmideen HN. In vitro- and in vivo-produced male dairy calves show molecular differences in the hepatic and muscular energy regulation. Biol Reprod. 2022;107(4):1113-24. http://doi.org/10.1093/biolre/ioac131. PMid:35766406.

Raes A, Wydooghe E, Pavani KC, Bogado Pascottini O, van Steendam K, Dhaenens M, Boel A, Heras S, Heindryckx B, Peelman L, Deforce D, van Nieuwerburgh F, Opsomer G, van Soom A, Smits K. Cathepsin-L secreted by high-quality bovine embryos exerts an embryotrophic effect in vitro. Int J Mol Sci. 2023;24(7):6563. http://doi.org/10.3390/ijms24076563. PMid:37047535.

Rivera RM, Goldkamp AK, Patel BN, Hagen DE, Soto-Moreno EJ, Li Y, Kim CN, Miller C, Williams F 3rd, Jannaman E, Xiao Y, Tribulo P, Estrada-Cortés E, Brau-Rodríguez AR, Hansen PJ, Wu Z, Spinka CM, Martin N, Elsik CG. Identification of large offspring syndrome during pregnancy through ultrasonography and maternal blood transcriptome analyses. Sci Rep. 2022;12(1):10540. http://doi.org/10.1038/s41598-022-14597-w. PMid:35732675.

Rizos D, Ward F, Duffy P, Boland MP, Lonergan P. Consequences of bovine oocyte maturation, fertilization or early embryo development in vitro versus in vivo: implications for blastocyst yield and blastocyst quality. Mol Reprod Dev. 2002;61(2):234-48. http://doi.org/10.1002/mrd.1153. PMid:11803560.

Sang L, Ortiz W, Xiao Y, Estrada-Cortes E, Jannaman EA, Hansen PJ. Actions of putative embryokines on development of the preimplantation bovine embryo to the blastocyst stage. J Dairy Sci. 2020;103(12):11930-44. http://doi.org/10.3168/jds.2020-19068. PMid:33041033.

Sang L, Xiao Y, Jiang Z, Forde N, Tian XC, Lonergan P, Hansen PJ. Atlas of receptor genes expressed by the bovine morula and corresponding ligand-related genes expressed by uterine endometrium. Mol Reprod Dev. 2021;88(10):694-704. http://doi.org/10.1002/mrd.23534. PMid:34596291.

Santos GMGD, Junior LB, Silva-Santos KC, Ayres Dias JH, Dias IDS, Seneda MM, Morotti F. Conception rate and pregnancy loss in fixed-time cattle embryo transfer programs are related to the luteal blood perfusion but not to the corpus luteum size. Theriogenology. 2023;210:251-5. http://doi.org/10.1016/j.theriogenology.2023.07.039. PMid:37549464.

Sciorio R, Miranian D, Smith GD. Non-invasive oocyte quality assessment. Biol Reprod. 2022;106(2):274-90. http://doi.org/10.1093/biolre/ioac009. PMid:35136962.

Seekford ZK, Wooldridge LK, Dias NW, Timlin CL, Sales ÁF, Speckhart SL, Pohler KG, Cockrum RR, Mercadante VRG, Ealy AD. Interleukin-6 supplementation improves post-transfer embryonic and fetal development of in vitro-produced bovine embryos. Theriogenology. 2021;170:15-22. http://doi.org/10.1016/j.theriogenology.2021.04.004. PMid:33957485.

Siqueira LGB, Dikmen S, Ortega MS, Hansen PJ. Postnatal phenotype of dairy cows is altered by in vitro embryo production using reverse X-sorted semen. J Dairy Sci. 2017;100(7):5899-908. http://doi.org/10.3168/jds.2016-12539. PMid:28456408.

Soares ACS, Lodde V, Barros RG, Price CA, Luciano AM, Buratini J. Steroid hormones interact with natriuretic peptide C to delay nuclear maturation, to maintain oocyte-cumulus communication and to improve the quality of in vitro-produced embryos in cattle. Reprod Fertil Dev. 2017;29(11):2217-24. http://doi.org/10.1071/RD16320. PMid:28356185.

Strączyńska P, Papis K, Morawiec E, Czerwiński M, Gajewski Z, Olejek A, Bednarska-Czerwińska A. Signaling mechanisms and their regulation during in vivo or in vitro maturation of mammalian oocytes. Reprod Biol Endocrinol. 2022;20(1):37. http://doi.org/10.1186/s12958-022-00906-5. PMid:35209923.

Suh RS, Zhu X, Phadke N, Ohl DA, Takayama S, Smith GD. IVF within microfluidic channels requires lower total numbers and lower concentrations of sperm. Hum Reprod. 2006;21(2):477-83. http://doi.org/10.1093/humrep/dei323. PMid:16199424.

Thibier M. Data Retrieval Committee statistics of Embryo Transfer – Year 2008. The worldwide statistics of embryo transfers in farm animals. Embryo Transfer Newsletter. 2009;27(4):13-9.

Tríbulo P, Jumatayeva G, Lehloenya K, Moss JI, Negrón-Pérez VM, Hansen PJ. Effects of sex on response of the bovine preimplantation embryo to insulin-like growth factor 1, activin A, and WNT7A. BMC Dev Biol. 2018;18(1):16. http://doi.org/10.1186/s12861-018-0176-2. PMid:30055575.

Trigal B, Gómez E, Díez C, Caamaño JN, Martín D, Carrocera S, Muñoz M. In vitro development of bovine embryos cultured with activin A. Theriogenology. 2011;75(3):584-8. http://doi.org/10.1016/j.theriogenology.2010.09.010. PMid:21040964.

Tšuiko O, Catteeuw M, Zamani Esteki M, Destouni A, Bogado Pascottini O, Besenfelder U, Havlicek V, Smits K, Kurg A, Salumets A, D'Hooghe T, Voet T, van Soom A, Robert Vermeesch J. Genome stability of bovine in vivo-conceived cleavage-stage embryos is higher compared to in vitro-produced embryos. Hum Reprod. 2017;32(11):2348-57. http://doi.org/10.1093/humrep/dex286. PMid:29040498.

Tutt DAR, Guven-Ates G, Kwong WY, Simmons R, Sang F, Silvestri G, Canedo-Ribeiro C, Handyside AH, Labrecque R, Sirard MA, Emes RD, Griffin DK, Sinclair KD. Developmental, cytogenetic and epigenetic consequences of removing complex proteins and adding melatonin during in vitro maturation of bovine oocytes. Front Endocrinol. 2023;14:1280847. http://doi.org/10.3389/fendo.2023.1280847. PMid:38027209.

Tutt DAR, Silvestri G, Serrano-Albal M, Simmons RJ, Kwong WY, Guven-Ates G, Canedo-Ribeiro C, Labrecque R, Blondin P, Handyside AH, Griffin DK, Sinclair KD. Analysis of bovine blastocysts indicates ovarian stimulation does not induce chromosome errors, nor discordance between inner-cell mass and trophectoderm lineages. Theriogenology. 2021;161:108-19. http://doi.org/10.1016/j.theriogenology.2020.11.021. PMid:33307428.

Urrego R, Bernal-Ulloa SM, Chavarría NA, Herrera-Puerta E, Lucas-Hahn A, Herrmann D, Winkler S, Pache D, Niemann H, Rodriguez-Osorio N. Satellite DNA methylation status and expression of selected genes in Bos indicus blastocysts produced in vivo and in vitro. Zygote. 2017;25(2):131-40. http://doi.org/10.1017/S096719941600040X. PMid:28137339.

Vallet-Buisan M, Mecca R, Jones C, Coward K, Yeste M. Contribution of semen to early embryo development: fertilization and beyond. Hum Reprod Update. 2023;29(4):395-433. http://doi.org/10.1093/humupd/dmad006. PMid:36882116.

VanRaden PM. Symposium review: how to implement genomic selection. J Dairy Sci. 2020;103(6):5291-301. http://doi.org/10.3168/jds.2019-17684. PMid:32331884.

Vargas LN, Nochi ARF, de Castro PS, Cunha ATM, Silva TCF, Togawa RC, Silveira MM, Caetano AR, Franco MM. Differentially methylated regions identified in bovine embryos are not observed in adulthood. Anim Reprod. 2023;20(1):e20220076. http://doi.org/10.1590/1984-3143-ar2022-0076. PMid:36938311.

Viana JHM. 2022 statistics of embryo production and transfer in domestic farm animals: the main trends for the world embryo industry still stand. Embryo Transfer Newsletter. 2023;41:20-38.

Viuff D, Rickords L, Offenberg H, Hyttel P, Avery B, Greve T, Olsaker I, Williams JL, Callesen H, Thomsen PD. A high proportion of bovine blastocysts produced in vitro are mixoploid. Biol Reprod. 1999;60(6):1273-8. http://doi.org/10.1095/biolreprod60.6.1273. PMid:10330080.

Willett EL, Black WG, Casida LE, Stone WH, Buckner PJ. Successful transplantation of a fertilized bovine ovum. Science. 1951;113(2931):247. http://doi.org/10.1126/science.113.2931.247.a. PMid:14809298.

Wooldridge LK, Ealy AD. Interleukin-6 promotes primitive endoderm development in bovine blastocysts. BMC Dev Biol. 2021;21(1):3. http://doi.org/10.1186/s12861-020-00235-z. PMid:33430761.

Wooldridge LK, Johnson SE, Cockrum RR, Ealy AD. Interleukin-6 requires JAK to stimulate inner cell mass expansion in bovine embryos. Reproduction. 2019;158(4):303-12. http://doi.org/10.1530/REP-19-0286. PMid:31408846.

Yaghoobi M, Abdelhady A, Favakeh A, Xie P, Cheung S, Mokhtare A, Lee YL, Nguyen AV, Palermo G, Rosenwaks Z, Cheong SH, Abbaspourrad A. Faster sperm selected by rheotaxis leads to superior early embryonic development in vitro. Lab Chip. 2024;24(2):210-23. http://doi.org/10.1039/D3LC00737E. PMid:37990939.

Yang QE, Fields SD, Zhang K, Ozawa M, Johnson SE, Ealy AD. Fibroblast growth factor 2 promotes primitive endoderm development in bovine blastocyst outgrowths. Biol Reprod. 2011;85(5):946-53. http://doi.org/10.1095/biolreprod.111.093203. PMid:21778141.

Zhang M, Zhang J, Wang D, Liu Z, Xing K, Wang Y, Jiao M, Wang Y, Shi B, Zhang H, Zhang Y. C-X-C motif chemokine ligand 12 improves the developmental potential of bovine oocytes by activating SH2 domain-containing tyrosine phosphatase 2 during maturation†. Biol Reprod. 2023;109(3):282-98. http://doi.org/10.1093/biolre/ioad079. PMid:37498179.

Zhu L, Marjani SL, Jiang Z. The epigenetics of gametes and early embryos and potential long-range consequences in livestock species-filling in the picture with epigenomic analyses. Front Genet. 2021;12:557934. http://doi.org/10.3389/fgene.2021.557934. PMid:33747031.

Author contributions

The author conceived of the paper, wrote it and edited it.