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Recent Advances and Emerging Applications in Embryo Vitrification for Livestock

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ABSTRACT

Vitrification has progressively supplanted conventional slow freezing as the method of choice for embryo cryopreservation in both human medicine and animal production, particularly for in vitro-produced embryos and for species or developmental stages highly sensitive to conventional freezing. By combining very high cooling and warming rates with concentrated cryoprotectant solutions and minimal sample volumes, it largely prevents intracellular ice formation and, in experienced hands, consistently yields post-warming survival and developmental rates that exceed those obtained with traditional controlled-rate freezing. In this review, we synthesize recent technical and biological advances in embryo vitrification, with particular attention to cryoprotectant formulations, carrier devices, cooling-rate control and current strategies to enhance cryotolerance. For the main livestock species—cattle, horses, pigs and small ruminants—we outline the present state of this technology, highlighting where vitrification has become routine practice and where adoption remains limited or experimental. Despite the clear progress achieved, implementation in field conditions is still heterogeneous, and truly standardized, species-adapted protocols—ideally amenable to automation and direct transfer—are yet to be fully established.

1 | Introduction

The long-term preservation of embryos in liquid nitrogen has become a cornerstone of assisted reproductive technologies in livestock animal species (Cuello, Gonzalez-Plaza, et al. 2024). In addition to its role in embryo transfer, cryobanking allows genetic conservation programmes and facilitates the international exchange of high-value germplasm, which is the basis of large-scale commercial breeding in bovine and equine (Lonergan 2024; Mogas et al. 2024; E. Squires 2020). Historically, embryo cryopreservation has been based on two principal methodologies: slow (or traditional) freezing and vitrification (Saragusty and Arav 2011).

From the 1970s until the early 2000s, slow freezing represented the standard reference technique for embryo cryopreservation. Slow freezing methods are based on a progressive dehydration of embryonic cells and controlled formation of extracellular ice, by using low concentrations of cryoprotectants and low cooling rates. However, in practice, slow freezing does not eliminate the risk of intracellular crystallization. This weakness is more evident in embryos with a high lipid content or large blastomeres, where heterogeneous cooling and phase transitions make them highly susceptible to cryoinjury (Marsico et al. 2019).

Vitrification follows a different approach to embryo cryopreservation. First described for mammalian embryos in the 1980s (Rall and Fahy 1985), it converts the aqueous phase into a glass-like amorphous solid rather than an ordered ice lattice. To accomplish this, cells are exposed to high concentrations of cryoprotective agents (CPAs) that increase solution viscosity and are then subjected to ultra-rapid cooling—typically at rates greater than 10,000°C/min—so that ice nucleation is effectively suppressed

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(Kuwayama et al. 2005; Vajta et al. 1997). The concept became practical in routine laboratories with the introduction, during the 2000s and 2010s, of minimal-volume carrier devices such as the Open Pulled Straw (OPS; Vajta et al. 1997), Cryotop (Kuwayama et al. 2005), Cryolock (El-Danasouri and Selman 2001), and Hollow Fibre Vitrification (HFV; Matsunari et al. 2012) system, among others (Kamoshita et al. 2024). Since then, vitrification has become a routine technique for the cryopreservation of human oocytes and embryos (Casciani et al. 2023; Nagy et al. 2020), it is progressively increasing its use for in vitro produced (IVP) embryos in cattle (Mogas et al. 2024), and it is the method of choice for in vivo produced embryos in most domestic animal species (Almiñana and Cuello 2015; Cuello, Gonzalez-Plaza, et al. 2024).

The applicability of the technique across livestock species, together with an overview of the most effective carrier systems and cryoprotectant formulations, has been recently reviewed by Kamoshita et al. (2024) and Cuello, Gonzalez-Plaza, Parrilla, et al. (2024) in the context of a major reference work on cryopreservation in assisted reproduction. This review examines recent innovations in vitrification protocols and devices, emerging strategies to improve the embryo viability after vitrification and warming, emerging applications of embryo vitrification and its status in livestock species. This narrative review focuses primarily on studies published in the last 10 years, identified through searches of major bibliographic databases indexing journals listed in the Journal Citation Reports, with additional articles selected based on their relevance to embryo vitrification in livestock.

2 | Strategies Based on the Physical Principles of Vitrification

For vitrification to succeed, the entire cell must pass through the glass transition temperature without ice nucleation during cooling and without devitrification (loss of the vitreous state and ice crystals formation) during warming (Saragusty and Arav 2011). Two interrelated variables determine whether this condition is fulfilled: the nature and concentration of the CPAs and the cooling and warming rates, which depend on the volume of solution surrounding the embryo.

2.1 | Cryoprotective Agents

The permeability of the plasma membrane and zona pellucida to water and CPAs is one of the most important factors determining the optimal conditions for embryo vitrification (Edashige 2017). Ethylene glycol (EG) is the permeating CPA of choice in most livestock species because of its relatively low toxicity and rapid membrane permeation; EG is often combined with dimethyl sulfoxide (Cuello, Gonzalez-Plaza, et al. 2024). Permeating CPAs cross the plasma membrane, replace intracellular water, and increase cytoplasmic viscosity. Non-permeating CPAs, such as sucrose, trehalose, polyvinylpyrrolidone, and Ficoll, remain outside the cell and promote osmotic dehydration, thereby reinforcing the vitrified state (Yong et al. 2020). In practice, mixtures of the two types of CPAs are used because combining CPAs reduces the required concentration of each component, which limits toxicity (Yong et al. 2020). Notable exceptions include the successful vitrification of pronuclear-stage porcine embryos using carboxylated ε-poly-L-lysine (COOH-PLL), which achieved 19.4% blastocyst development and resulted in the birth of 15 healthy piglets after transfer (Kamoshita et al. 2017). Additionally, emerging research has explored the use of antifreeze proteins in IVP bovine embryo vitrification, with AFP from *Lolium perenne* (LpAFP) improving hatching rates and total cell numbers while mitigating intracellular damage (Silva Júnior et al. 2023), as well as exopolysaccharides such as in bovine IVP embryos, which significantly enhanced post-warming re-expansion and hatching rates while reducing apoptotic gene expression (Martínez-Rodero et al. 2022).

2.2 | Vitrification Devices and Cooling Rate

The recognition that cooling rate increases as the volume of vitrification solution decreases led to the development of minimal-volume open carrier devices. Open systems, such as Cryotop or Superfine OPS (SOPS), achieve ultra-rapid cooling rates (> 20,000°C/min) by exposing embryos in minimal volumes (< 1 μL) directly to liquid nitrogen, resulting in excellent post-warming survival rates in porcine, bovine, and equine embryos. Later heat-transfer modelling revealed that cooling rates can vary by more than an order of magnitude across devices, reaching approximately 180,000°C/min for the nylon cryoloop, 37,500°C/min for the Cryotop, and 5500°C/min for the OPS under comparable conditions (Sansinena et al. 2011). In open systems, the vitrification medium comes into direct contact with liquid nitrogen, maximizing the cooling rate; however, debate exists regarding the risk of pathogen contamination (Cuello et al. 2025; Molina et al. 2016). Despite this theoretical risk, no disease transmission has been reported in humans or domestic animals following around 600,000 to 1,000,000 transfers worldwide using open vitrification systems (Bielanski 2012; Vajta and Nagy 2006), and experimental studies confirm that contamination does not occur using proper vitrification and storage protocols (Cuello et al. 2025). In contrast, closed systems generally achieve lower post-warming survival rates due to reduced cooling and warming rates (Cai et al. 2018; Vajta et al. 2015). This trade-off continues to favour open systems such as Cryotop and OPS for livestock embryo cryopreservation (Cuello, Gonzalez-Plaza, et al. 2024).

In polytocous species, the simultaneous vitrification of many embryos is essential to facilitate embryo transfer under field conditions. However, achieving the simultaneous vitrification of multiple embryos while maintaining the principle of minimum-volume vitrification remains a limitation of most vitrification devices. Recently a purpose-built carrier has been developed: a 3D-printed CryoEyelet device was validated in rabbits (Marco-Jiménez et al. 2023), and the modified Open Cryotop system has been shown to load at least 20 porcine morulae and blastocysts per device without compromising viability compared with fresh controls (Gonzalez-Plaza et al. 2022).

3 | Simplification of Procedures Using Automated Vitrification and Direct Warming Systems

A limitation of vitrification technology is its dependence on the embryologist's manual skill and experience, which introduces operator-dependent variability (Arav et al. 2018). In human assisted reproduction, fully automated platforms such as Gavi

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(Roy et al. 2014) and semi-automated systems like SHARA (Arav et al. 2018) have been successfully implemented, achieving outcomes comparable to manual methods. Automated platforms based on microfluidics have also been successfully applied with mouse embryos (Lin et al. 2025). However, their application in livestock remains limited and primarily experimental. Arav et al. (2018) tested the automated device SHARA with bovine zygotes, achieving only 9% of blastocyst formation rate. Currently, manual methods such as Cryotop and SOPS remain the standard for vitrification in domestic animals (Cuello, Gonzalez-Plaza, et al. 2024) but extending automated platforms to livestock embryos could finally enable the standardized; the field has been aiming for operator-independent embryonic cryopreservation.

The development of one-step warming systems or in-straw warming for direct embryo transfer makes the technology more practical for field applications in livestock. In cattle, in-straw warming of IVP embryos has similar survival to conventional multi-step warming (Caamaño et al. 2015), with pregnancy rates comparable to fresh embryos (Gonzalez-Rodríguez et al. 2022; Oliveira et al. 2020). Piglets have been obtained after surgical (Cuello et al. 2016) or non-surgical transfer (Martinez et al. 2015) of vitrified embryos warmed using one-step protocols. Recent studies have also described excellent pregnancy rates using one-step warming in equine (Couto et al. 2025) and ovine (Islam et al. 2025) embryos. One of the most highly innovative lines of research is volumetric nano-warming, which aims to achieve sufficiently high and uniform warming rates to prevent devitrification. This topic of research has been recently reviewed by Ayantoye et al. (2026).

4 | Targeted Strategies to Improve Vitrification Efficiency and Outcomes

Recent advances in livestock embryo vitrification have focused on three main strategies to improve cryotolerance and post-warming survival across species. First, antioxidant supplementation during embryo culture has emerged as the most widely studied approach. In cattle, recent investigations confirm that compounds such as resveratrol (Saber et al. 2024), melatonin (Wang et al. 2014), or tauroursodeoxycholic acid (TUDCA; Pioltine et al. 2023) significantly reduce oxidative stress and apoptosis while improving viability of vitrified-warmed embryos. Second, modulation of embryonic lipid content using forskolin has proved particularly useful in pigs and IVP embryos, which are characterized by an exceptionally high load of cytoplasmic lipid droplets. The short-term exposure of embryos to forskolin has been shown to stimulate cAMP-dependent lipolysis, and consequently improved the cryotolerance of very early in vivo derived porcine embryos, increasing blastocyst formation after vitrification and warming (Gomis et al. 2013). In cattle, combined supplementation of the in vitro culture medium with forskolin and linoleic acid has been reported to remodel lipid droplets and improve the survival of vitrified embryos, suggesting that fine-tuning fatty-acid metabolism can mitigate some of the intrinsic sensitivity of IVP embryos to cryoinjury (Zhang et al. 2025).

Third, novel mechanical approaches aimed at reducing the volume and hydrostatic tension of the blastocoel, such as artificial cavity collapse by aspiration of blastocoelic fluid immediately before vitrification, have yielded strikingly positive results in cattle (Martínez-Rodero, Salas-Huetos, et al. 2024). Expanded blastocysts subjected to fluid aspiration show post-warming re-expansion and hatching rates like fresh controls and are clearly superior to embryos that are vitrified intact or following trophectoderm biopsy, indicating that controlled blastocoel collapse can substantially improve cryotolerance without adding significant manipulation-related damage (Martínez-Rodero, Salas-Huetos, et al. 2024).

In equine embryos, micromanipulation to collapse the blastocoel before vitrification has enabled successful cryopreservation of expanded blastocysts up to 650-μm diameter, with pregnancy rates > 75% for embryos 300–550 μm (E. L. Squires 2016; Umair et al. 2023).

Another line of work involves supplementing IVP culture media with extracellular vesicles (EVs) derived from oviductal and uterine fluids; this strategy has improved bovine blastocyst quality and post-vitrification survival (Leal et al. 2022). Emerging approaches also include modulators of aquaporin expression such as pioglitazone (Zhang et al. 2026) or furosemide (Min et al. 2025) that have been shown to improve bovine embryo dehydration and cryosurvival. Among these approaches, blastocoel collapse and lipid modulation appear closest to practical implementation, whereas EV supplementation and aquaporin modulation remain promising but less validated.

5 | Mathematical Models and Artificial Intelligence in Embryo Vitrification

The integration of mathematical models and artificial intelligence (AI) into embryo vitrification is an emerging field with considerable potential. Mathematical modelling of membrane permeability has been applied to the rational design of vitrification protocols for bovine oocytes and in vitro-produced embryos, enabling the prediction of optimal cryoprotectant exposure times and concentrations (Mogas et al. 2024), while the use of AI for embryo quality assessment was identified as a promising avenue to further improve vitrification outcomes (Mogas et al. 2024). Building on this, deep learning models have demonstrated the ability to predict blastocyst survival after warming using time-lapse imaging captured in the first hours post-warming (Marsh et al. 2022). Despite this progress, the direct application of AI and computational tools to the optimization and standardization of embryo vitrification protocols across livestock species remains virtually unexplored, representing a significant gap in the current literature.

6 | Emerging Applications of Embryo Vitrification in Animal Reproduction

Embryo vitrification is becoming increasingly relevant for the cryobanking of genome-edited, cloned and transgenic livestock lines. As CRISPR/Cas9 and related gene-editing approaches become more widely applied in livestock, there is an increasing need to safeguard edited and cloned lines through reliable cryobanking of embryos (Dos Santos-Neto et al. 2023; Haraguchi et al. 2024; Nguyen et al. 2023). Work in pigs and cattle has shown that cloned or transgenic embryos can withstand vitrification and retain the capacity to develop to blastocyst and term, although usually with some penalty in developmental rates compared with fresh

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counterparts (Du et al. 2007; Gong et al. 2004; Hou et al. 2005; Jia et al. 2020; Li et al. 2006; Lonergan et al. 2007; Yang et al. 2008). In pigs, proof-of-concept work has shown that very early cloned embryos and delipidated transgenic blastocysts generated by somatic cell nuclear transfer can be vitrified on carriers such as Cryotop or OPS and later establish pregnancies resulting in live piglets (Du et al. 2007; Jia et al. 2020; Li et al. 2006). These studies demonstrate that banking and even international movement of genetically modified porcine embryos are technically possible using an appropriate combination of delipidation and an adequate vitrification protocol.

Comparable investigations in cattle indicated that OPS-vitrified oocytes retain sufficient competence to act as nuclear recipients for SCNT and to support development to term, and also that cloned or genetically modified blastocysts survive vitrification and result in healthy calves, although with lower pregnancy rates and altered fetal growth reports compared with in vivo derived embryos (Gong et al. 2004; Hou et al. 2005; Lonergan et al. 2007; Yang et al. 2008). More recent studies that combine vitrification with electroporation-based CRISPR/Cas9 editing in bovine and porcine zygotes suggest that the use of vitrified zygotes for genome editing has little influence on editing efficiency, even though blastocyst yields remain always lower in vitrified than in fresh zygotes (Haraguchi et al. 2024; Somfai et al. 2023). Collectively, these findings support vitrification as a practical—though still inefficient—strategy for banking cloned and genome-edited livestock embryos. The demand for an optimal approach for the vitrification of these edited or cloned embryos makes necessary the optimization of vitrification protocols, the improvements of culture conditions, and investigating the combined effect of these technologies on embryo and foetal development.

7 | Embryo Vitrification: Species-Specific Considerations and Challenges

7.1 | Cattle

Among domestic species, cattle have seen the widest commercial uptake of embryo vitrification. Of the approximately 1.5 million bovine embryos transferred worldwide in 2021, close to 80% were produced in vitro (Krisher and Herrick 2024). In cattle, vitrification has been recommended as the best approach for cryopreserving IVP embryos, and also has become the context in which some of the most advanced strategies to improve cryotolerance are being explored (Ferré et al. 2020; Mogas et al. 2024). Biophysical and in silico models are now being used to optimize equilibration times and temperatures, leading to shorter, better-defined protocols that improve re-expansion and pregnancy rates (40%–50%; Table 1) of IVP blastocysts to levels comparable with fresh controls (Martínez-Rodero, Diaz-Muñoz, et al. 2024; Mogas et al. 2024). In addition, methodological reviews highlight how interventions such as modulating lipid content, limiting oxidative damage or refining CPA exposure protocols can significantly enhance the survival of vitrified bovine embryos (Mogas et al. 2024; Tu et al. 2022). As a result, the bovine model is currently at the forefront of efforts to refine embryo vitrification through targeted strategies and the use of non-invasive markers, quantitative modelling, and increasingly, artificial intelligence–based decision support systems.

7.2 | Horse

Vitrification of equine embryos is technically challenging, and it is constrained by blastocyst diameter (Hinrichs 2020; Stout 2012). Expanded blastocysts are large (often $> 300\,\mu m$) and surrounded by a glycoprotein capsule, which makes them intrinsically difficult to dehydrate and permeate with CPAs (Scott et al. 2012). Embryos recovered in vivo with diameters below about $300\,\mu m$ can be vitrified using standard minimal-volume protocols, but larger embryos usually require preliminary blastocoel collapse, achieved by microaspiration, to reduce volume and facilitate CPA penetration (Wilsher et al. 2020); with this approach, non-surgical transfer yields pregnancy rates in the order of 50%–60% (Choi and Hinrichs 2017; E. Squires 2020). Over the last decade, one of the most significant advances in equine reproduction has been the widespread adoption of ovum pick-up combined with conventional in vitro fertilization or ICSI (Canesin et al. 2020; Choi and Hinrichs 2017; Martin-Pelaez et al. 2025; Morris and

TABLE 1 | Pregnancy and farrowing rates following transfer of vitrified in vitro-produced bovine blastocysts. Data were collected from publications over the last 5 years.

Kind of blastocyst	Vitrification method	Transfers (*N*)	Pregnancy rate (%)	Farrowing rate (%)	References
Full Bl	Cryotop	20	35.0	—	Gonzalez-Rodríguez et al. (2022)
	VitTrans	15	33.3		
Early Bl	Cryotop	34	17.6	—	Reviewed by Valente et al. (2022)
Full Bl	Cryotop	1453	38.7	—	
Expanded Bl	Cryotop	5536	43.7	—	
Blastocyst	Cryotop	155	50.9	—	Kim and Yi (2023)
Cloned Bl	Cryotop	56	41.4–60.0[a]	14.6–26.7[a]	Su et al. (2025)
Full Bl	OPS	3669	34.6	—	Amorim da Silva Coelho et al. (2025)
Full Bl	OPS	150	33.3–56[a]	—	Martins et al. (2025)

Abbreviations: Bl, blastocyst; OPS, open pulled straw.
[a]Range of pregnancy rates across the experimental groups.

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Maclellan 2024) for in vitro embryo production, together with vitrification for embryo storage prior to transfer. These advances have greatly promoted the expansion of the international equine embryo trade and improved the efficient use of recipient mares (Table 2). One of the most recent innovations has been the metabolomic profiling of Day 4 ICSI-derived embryos, enabling the identification of biomarkers of developmental competence and opening new opportunities for embryo selection prior to vitrification (Tsopp et al. 2026).

7.3 | Pig

The pig has long been regarded as one of the most challenging domestic species for embryo cryopreservation. Currently, vitrification remains the only practically effective approach for the long-term storage of porcine embryos (Gil et al. 2024; Martinez et al. 2016, 2019; Xingzhu et al. 2021). Notably, important progress has been made in recent years: vitrification of in vivo–derived morulae and blastocysts can now be performed without pretreatment and using chemically defined media, while achieving survival rates comparable to those of fresh control embryos and exerting minimal effects on embryo quality (Cuello et al. 2016; Cuello, Martinez, Cambra, Gonzalez-Plaza, et al. 2021; Cuello, Martinez, Cambra, Parrilla, et al. 2021; Cuello, Gonzalez-Plaza, et al. 2024). SOPS vitrification has successfully produced piglets after the transfer of vitrified-warmed morulae and blastocysts (Table 3), with acceptable fertility (~70%) and prolificacy (~9.5 piglets per litter) following either surgical (Cuello et al. 2016) or nonsurgical embryo transfer procedures (Gomis et al. 2012; Martinez et al. 2015).

In parallel, a series of technical developments has brought this technology closer to routine use under field conditions. These include the introduction of direct warming systems (Cuello, Gonzalez-Plaza, et al. 2024), modifications of the Cryotop device to enable the simultaneous vitrification of at least 20 embryos (Gonzalez-Plaza et al. 2022, 2023, 2024), and the combination of vitrification with non-surgical embryo transfer (Cuello et al. 2016; Gomis et al. 2012; Martinez et al. 2019). These improvements remove major obstacles to the commercial application of embryo transfer in pigs. Efficient vitrification of IVP embryos is increasingly demanded for the banking of pig biomodels (Kikuchi et al. 2016). Regarding the cryopreservation of oocytes or zygotes for gene editing purposes, it has been shown that the use of the zygote stage is preferable (Haraguchi et al. 2024; Somfai et al. 2023). Progress has been made also in the vitrification of IVP blastocysts (Gonzalez-Plaza et al. 2024;

TABLE 2 | Pregnancy and farrowing rates following transfer of vitrified equine blastocysts. Data were collected from publications over the last 10 years.

Embryo source	Kind of blastocyst	Vitrification method	Transfers (*N*)	Pregnancy rate (%)	Farrowing rate (%)	References
IVP	Blastocyst	MPTs	47	61.7	36.2	Choi and Hinrichs (2017)
In vivo	Bl < 300 μm	MPTs/Sujo	21	76.5/75.0	70.6/50.0	
In vivo	Bl > 300 μm	MPTs/Sujo	15	45.5/25.0	45.5/25.0	
In vivo	Bl ≤ 550 μm[b]	Cryolock	12	75.0	—	Wilsher et al. (2019)
	Bl ≤ 550 μm[c]		10	80.0		
	Bl > 550 μm[b]		10	10.0		
	Bl > 550 μm[c]		18	72.2		
IVP	Blastocyst	Cryolock	30	83.3	—	Canesin et al. (2020)
In vivo	Bl ≤ 500 μm	Cryolock	31	80.6	—	Kovacsy et al. (2024)
	Bl > 500 μm		7	0.0		
IVP	Blastocyst	OPS	145	69.0	—	Morris and Maclellan (2024)
In vivo	Bl > 300 μm[c]		109	56.9		
In vivo	Bl ≤ 300 μm	Cryolock	59	85.7–88.9[a]	—	Couto et al. (2025)
	Bl > 300–400 μm		34	68.1–83.3[a]		
	Bl > 400–500 μm		24	0.0–78.6[a]		
IVP	Blastocyst	Cryolock	41	66.7–100[a]	—	Martin-Pelaez et al. (2025)
In vivo	Bl ≤ 400 μm	Cryolock	67	82.9–84.6[a]	—	Wilsher et al. (2026)
	Bl > 400 μm		42	17.9–71.4[a]		

Abbreviations: Bl, blastocyst; MPTs, micropipettes tips; OPS, open pulled straw.
[a]Range of pregnancy rates across the experimental groups.
[b]Punctured.
[c]Collapsed.

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TABLE 3 | Pregnancy and farrowing rates following transfer of vitrified porcine, ovine and caprine embryos. Data were collected from publications over the last 10 years.

Specie	Embryo source	Stage	Vitrification method	*N*	TE/recipient	Farrowing rate (%)	References
Porcine	In vivo	Mo/Bl	SOPS	14	25	71.4	Cuello et al. (2016)
Porcine	In vitro	PN	Cryotop	8	97–143	25.0	Kamoshita et al. (2017)
Porcine	In vivo	Mo	Metal mesh	5	20	20.0	Nakamura and Kikuchi (2017)
Porcine	In vivo	Mo/Bl	OPS	7	20–23	57.1	Tajima et al. (2020)
Porcine	In vivo	Bl	MVAC	12	15	66.7	Hirayama et al. (2020)
						66.7	
Caprine	In vitro	Bl	OPS	40	2	15.0–45.0[a]	Ferreira-Silva et al. (2017)
Caprine	In vitro	SCNT-Bl	Cryotop	22	3–4	36.4	Hajian et al. (2020)
Caprine	In vivo	Mo/Bl	S-VitriTip	38	1–2	50.0	Bruno-Galarraga et al. (2025)
Ovine	In vivo	GM-PN stage	OPS	14	8–9	21.4	Tian et al. (2018)
Ovine	In vivo	Mo/Bl	S-VitriTip	25	1–2	15.9	Figueira et al. (2019)
Ovine	In vivo	Mo	S-VitriTip	60	1	100	Gibbons et al. (2019)
		Bl		33			
		Bl		40			
Ovine	In vitro	GM-Bl	Cryotop	159	3	30.8	Dos Santos-Neto et al. (2023)
Ovine	In vivo	2–8 cells	OPS	12	2	50.0	Selionova et al. (2023)
		Mo/Bl		12		50.0	
Ovine	In vivo	Mo/Bl	OPS	46	2	43.3–50.0[a]	Islam et al. (2025)

Abbreviations: Bl, blastocyst; GM, genetically modified; Mo, morulae; MVAC, microvolume air cooling; *N*, number of embryo transfers; OPS, open pulled straw; SCNT, somatic cell nuclear transfer; TE, transferred embryos.
[a]Range of pregnancy rates across the experimental groups.

Morató et al. 2016; Nohalez et al. 2018) and cloned transgenic embryos (Du et al. 2007; Li et al. 2006); however, the efficient vitrification of this kind of embryo is still a challenge.

7.4 | Sheep and Goats

In sheep and goats, in vivo derived morulae and blastocysts show high tolerance to vitrification, achieving consistent pregnancy and farrowing rates under field conditions (Table 3). In sheep, minimal-volume systems such as OPS, Cryotop/Cryotip, and VitriTip applied to Day 7 embryos have produced lambing rates ranging from 50% to nearly 100% per recipient, and around 50% even at earlier developmental stages when embryo quality and transfer conditions are optimized (Dos Santos-Neto et al. 2017; Figueira et al. 2019; Gibbons et al. 2019; Islam et al. 2025; Selionova et al. 2023). Vitrification has also been successfully integrated into transgenic programs, enabling cryobanking of pronuclear embryos and yielding lambing rates of 30%–35% with IVP blastocysts (Dos Santos-Neto et al. 2023).

In goats, although outcomes are more variable, promising results have been reported, including kidding rates of ~45% with OPS-vitrified in vitro blastocysts, 30%–40% pregnancy rates in cloned embryos vitrified with Cryotop, and around 50% with in vivo embryos using S VitriTip (Bruno-Galarraga et al. 2025; Ferreira-Silva et al. 2017; Hajian et al. 2020). Overall, ovine embryos, especially those derived in vivo, are more tolerant to vitrification than caprine embryos; however, advances in minimal-volume devices and cryoprotectant protocols have reduced interspecies differences, supporting routine or semi-routine application in small ruminant reproduction and biotechnology (Ferreira-Silva et al. 2017).

8 | Conclusions and Future Perspectives

Cryopreservation continues to be one of the most impactful advances in animal reproductive technologies. There is still a gap between laboratory success and field-scale reproducibility that continues to limit the full impact of this approach on livestock reproduction. The heterogeneity of devices and protocols represents another important obstacle to the widespread adoption of embryo vitrification in livestock species. However, several overlapping developments are now guiding the future of embryo vitrification. Integration of AI-driven algorithms with

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time-lapse should improve identification of embryos most likely to withstand vitrification in cattle and horses. The expanding use of CRISPR/Cas9 and related editing systems in livestock will increase demand for secure long-term storage of genetically modified embryos, encouraging further species-specific optimization of protocols. Novel cryoprotectants with reduced toxicity are being evaluated as alternatives or adjuncts to conventional CPAs cocktails. In addition, microfluidic platforms that automate CPAs loading and removal will be fully adapted to animal embryos, reducing the operator dependence and improving standardization. Altogether, these advances represent a significant step toward making vitrification a practical approach for commercial livestock breeding programs and an efficient tool for the banking of valuable genetics and biomodels.

In addition to the promising advances summarized above, several practical barriers still limit the widespread commercial implementation of embryo vitrification in livestock. Regulatory requirements for embryo handling and movement, the cost of specialized devices and consumables, and the need for continuous operator training remain major constraints, particularly for small and medium-sized operations. Furthermore, the coexistence of multiple, only partially harmonized protocols across laboratories hampers standardization and complicates technology transfer to the field. Addressing these issues through clearer regulatory frameworks, cost-effective devices, and validated, species-adapted standard operating procedures will be essential to fully exploit the potential of embryo vitrification in commercial settings.

Author Contributions

C.C. conceptualized the manuscript and wrote the original draft. All co-authors reviewed and edited the manuscript. C.C., M.A.G., I.P., and E.A.M. acquired external funding for the authors' own studies discussed in this review.

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Ethics Statement

Animal husbandry and experimental procedures, when applicable to the authors' own studies discussed in this review, were conducted in accordance with European Directive 2010/63/EU (22/09/2010) and Spanish (ES300130640127) legislation, and were approved by the corresponding ethical committees prior to the experiments.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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