CHAPTER II

LITERATURE REVIEWS

2.1 Assisted reproductive technologies (ART).

In cattle, ART including artificial insemination (AI), ovum pick up (OPU), *in vitro* embryo production (IVP), cryopreservation and embryo transfer (ET) constitute a potential pipeline to produce entire genetics, sex determination and assessment of genomic estimated breeding values (GEBVs) of embryos. This technique can be used to improve the quality of oocytes and embryos. (Najafzadeh et al., 2021)

2.2 *In vitro* embryo production (IVP) of bovine is composed of 3 steps

2.2.1 *In vitro* maturation (IVM)

It is widely known that oocytes with cumulus cells are important in the development of oocytes during IVM. So, it is important to allow low-quality oocytes to mature by staying on the cumulus cells. It has been reported that oocytes with cumulus cells cultured in tissue culture medium-199 (TCM-199) supplemented with FSH and cysteamine, can increase the growth rate and cleavage of oocytes. There are reported that the optimal duration for this procedure is 16-24 hours after initiation of IVM. (Atef et al., 2005)

2.2.2 *In vitro* fertilization (IVF)

Fertilization would be considered the most critical step for IVP technology in any species. Many factors may affect the efficiency of IVF, such as sperm viability and capability, optimum *in vitro* environment for survival of gametes, time of insemination, duration of co-incubation, the presence of cumulus cells and the acquisition of the oocyte developmental competence during the complicated process of cytoplasmic maturation. Tyrode's albumin lactate pyruvate (TALP) solution is supplemented with many agents, such as bovine serum albumin (BSA), heparin, caffeine, penicillamine, hypotaurine, and calcium ionophore. There are several methods utilized for sperm preparation, such as swim up and percoll gradient centrifugation before being fertilized with oocytes. (Galli et al., 2003)

2.2.3 *In vitro* culture (IVC)

In recent years, the remarkable improvement of blastocyst formation rate (35-40%) is attained principally due to the optimization of the IVM and in part of the IVF systems rather than to environmental modifications applied to the IVC system. Despite high blastocyst formation rates, pregnancy rates following ET of cryopreserved bovine embryos are very poor. Bovine embryos develop in several media, such as synthetic oviductal fluid (SOF) (Carolan et al., 1995), TCM-199 and Chrerrles Rozenkrans 1aa medium (CR1aa) (Somfai et al., 2010) supplemented with various development influencing agents like essential and non-essential amino acids, fetal calf serum (FCS), insulin and insulin-like growth factor-1 (IGF-1). The presence of oviductal cells in the culture medium during IVC of the embryo is found to be very important for enhancing its developmental potential. Subsequently, zygotes/embryos have been successfully cultured in both SOF and another chemically defined medium, known as potassium simplex optimized medium (KSOM) (Sirisathien, 2002) with similar embryo development. In the IVC medium, the cleavage rate is improved up to 72% and the blastocyst rate per cleavage up to 52%. To prevent the oxidative damage of DNA, some antioxidants like ascorbic acid and alpha-tocopherol could play an active role in the growth and development of cattle embryos by neutralizing free radicals. Recently, much higher concentrations of glucose in both IVM and IVC have been proven beneficial for blastocyst development. It is also reported that the addition of leukemia inhibitory factor to the IVC medium improves blastocyst development and quality (Rizos et al., 2008). An improvement in embryo quality indicated by faster development and increased cryotolerance, was determined by a high concentration of hyaluronic acid during late IVC (Bousquet et al., 1999).

2.3 The environment of culture bovine embryo

In vitro embryo production serves as an essential tool for investigating pre-implantation embryonic development. Normally, embryos produced within the body develop in oviduct and uterus. This is unlike embryos produced *in vitro* where inappropriate environmental factors result in differences in biochemical signals that affect the genome (Rizos et al., 2008). Given the high sensitivity of pre-implantation embryos to environmental factors, inadequacies in culture conditions often result in suboptimal development (Ahmadi et al., 2023; Feuer and Rinaudo, 2012). The culture of mammalian embryos *in vitro* necessitates an appropriate environment wherein the early embryo can

undergo multiple cleavage divisions and eventually develop into a blastocyst (Campbell, 2013; Rizos et al., 2002; Thompson, 1997).

Embryos at the pre-implantation stage can be cultured in various media, ranging from simple balanced salt solutions like CR1aa, SOF, and KSOM, to more complex compositions such as TCM-199, often supplemented with serum and/or a feeder layer of somatic cells (Niemann and Wrenzycki, 2000; Summers and Biggers, 2003). However, the most substantial decline in *in vitro* development occurs during the latter phase of the process (post-fertilization culture), which involves in spanning from the two-cell to the blastocyst stages (Rizos et al., 2008). Moreover, increased oxidative stress is another factor that hinders *in vitro* embryo development. Therefore, there is a compelling need to enhance and refine the culture system aiming to potentially enhance the development of cultured embryos. This can be achieved by antioxidants supplementing such as cysteamine, cysteine, β-mercaptoethanol (Nedambale et al., 2006), and resveratrol (Gaviria et al., 2019; Wang et al., 2019) to the IVC media.

2.4 Evaluation and classification of bovine embryos

The IETS grading and classification system for embryos involves the assessment of embryos based on their stage of development and quality. Embryos are typically recovered 6-8 days after the onset of estrus. The classification includes a two-digit code: the first digit indicates the stage of development, and the second digit represents the quality grade (Stringfellow and Seidel, 1998).

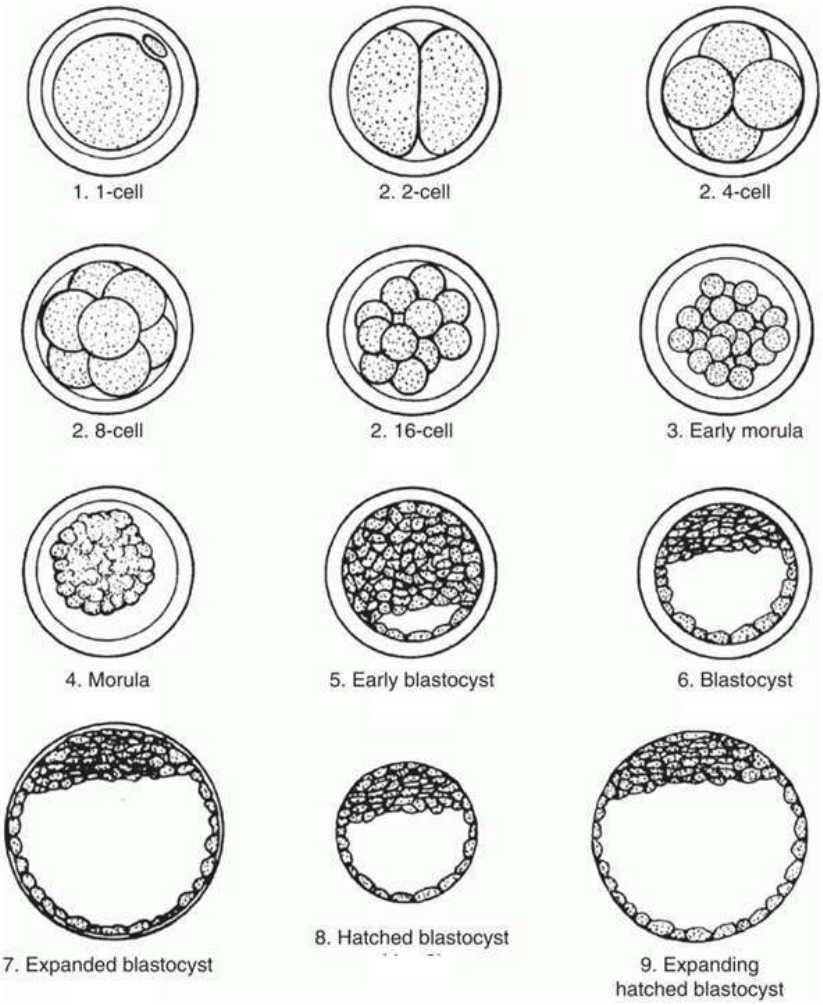

Figure 2.1 Normal embryonic development of bovine embryos (Stringfellow and Seidel, 1998).

Table 2.1 Stages of Embryonic Development (Stringfellow and Seidel, 1998).

Stage code	Name Stage code	Description
1	Unfertilized	A single cell, typically a UFO (unfertilized ovum).
2	2-cell to 12-cell	Contains 2-12 blastomeres, usually dead or degenerate.
3	Early morula	Group of at least 16 cells, not well-suited for cryopreservation.
4	Compact morula	Tightly compacted ball of cells, difficult to discern individual blastomeres.
5	Early blastocyst	Presence of a small fluid-filled cavity (blastocoele).
6	Blastocyst	Defined trophoblast layer, blastocoele cavity, and ICM cells.
7	Expanded blastocyst	Increased diameter and thinning of the zona pellucida.
8	Hatched blastocyst	Embryo undergoing or having completed hatching from the zona pellucida.
9	Expanded hatched blastocyst	Larger diameter than stage 8, uncommon to recover unless flushing is done more than 8 days after estrus.

Table 2.2 Quality grades of embryo (Stringfellow and Seidel, 1998).

Quality grade code	Name	Description
1	Excellent or good	Symmetrical, spherical embryo mass with uniform blastomeres; at least 85% viable cells.
2	Fair	Moderate irregularities in shape, size, color, and density of blastomeres; at least 50% viable cells.
3	Poor	Major irregularities in embryonic mass or blastomere characteristics; at least 25% viable cells.
4	Dead or degenerating	Nonviable entities, not suitable for transfer or cryopreservation.

2.5 Cryopreservation

Cryopreservation of embryos is widely used and plays an important role in the breeding and conservation of animal species. Therefore, technology must be developed that can effectively preserve embryos at low temperatures and maintain embryo survival. There are two methods of cryopreservation that are popular currently (Najafzadeh et al., 2021).

2.5.1 Cryoprotectant (CPAs)

Cryoprotectants (CPAs) are essential for the cryopreservation of bovine blastocysts to protect them from damage during the freezing and thawing processes. The use of CPAs enables the preservation of embryo viability and function post-thawing, which is crucial for successful embryo transfer and subsequent development.

Types of cryoprotectants: Cryoprotectants can be broadly classified into two categories: permeating and non-permeating.

Permeating cryoprotectants are essential for the successful cryopreservation of bovine blastocysts. These CPAs can penetrate the cell membrane and include substances such as Dimethyl Sulfoxide (DMSO), Glycerol, Ethylene Glycol (EG), and Propylene Glycol. DMSO is one of the most used CPAs, offering effective protection by

replacing water within the cells and preventing ice crystal formation. Glycerol is often used for its ability to permeate cells and reduce ice formation, although it can sometimes be toxic to embryos. Ethylene Glycol is widely used due to its relatively low toxicity and high permeability, making it effective for cryopreserving bovine embryos. Propylene Glycol is another permeating CPA, known for its compatibility with various embryo stages and relatively low toxicity. (Leibo and Pool, 2011)

Non-permeating cryoprotectants (CPAs) remain outside the cell and primarily work by dehydrating cells and forming a protective glass-like matrix during freezing. Examples include sucrose, which is often used in combination with permeating CPAs to enhance dehydration and reduce ice formation, and trehalose, which similarly helps to stabilize cell membranes and proteins during freezing. Polyvinylpyrrolidone (PVP) is another non-permeating CPA that creates a viscous environment, reducing ice formation and mechanical stress on cells. (Leibo and Pool, 2011)

High concentrations of cryoprotectants (CPAs), especially during vitrification, can be toxic to embryos, necessitating a careful balance of CPA concentration and exposure time. The permeability of CPAs varies, influencing the selection and combination of these agents. Different stages of embryonic development may require specific CPA protocols to achieve optimal cryopreservation outcomes. The effectiveness of cryopreservation protocols is ultimately measured by the post-thaw survival and developmental competence of the embryos. (Mazur, 1984; Rall and Fahy, 1985)

2.5.2 Slow freezing

It is a method for slowly freezing embryos. It will cause cold from the outside into the inside. It is an easy method that does not require much expertise, but it takes a longer time, after slow freezing and decreases the survival rate when compared with fresh embryos (Saragusty and Arav, 2011).

The success of slow freezing has been reported with the first calves born after embryo freezing (Fuku et al., 1992). Since then, it has progressed and developed a simpler and more stable protocol (Ferré et al., 2020).

2.5.3 Vitrification

Vitrification is a method of rapid freezing of embryos. It directly cools the embryo with a high concentration of cryoprotective agents (CPA) in the medium. It is a simple method and requires a lot of expertise but takes less time. Embryos can be frozen in large numbers; however, the embryos after being frozen in this way still have a low survival rate when compared with fresh embryos (Nedambale et al., 2006), but the survival rate is higher than slowly freezing technique (Najafzadeh et al., 2021). Previously report found that vitrified MII-stage oocytes have higher survival rates than GV-stage oocytes (Chaves et al., 2017).

Vitrification is a better alternative than slow freezing because of its cost-effectiveness, speed and simplicity (Mochida and Ogura, 2010). So, vitrification become to the routine method for freezing oocytes and embryos of human (Azawi et al., 2013; Dhali et al., 2019) along with freezing embryos in animals such as bovine (Mohr and Trounson, 1981), rabbits (Silvestre et al., 2002), horses (Massip, 2001) and pigs (Dobrinsky, 2002).

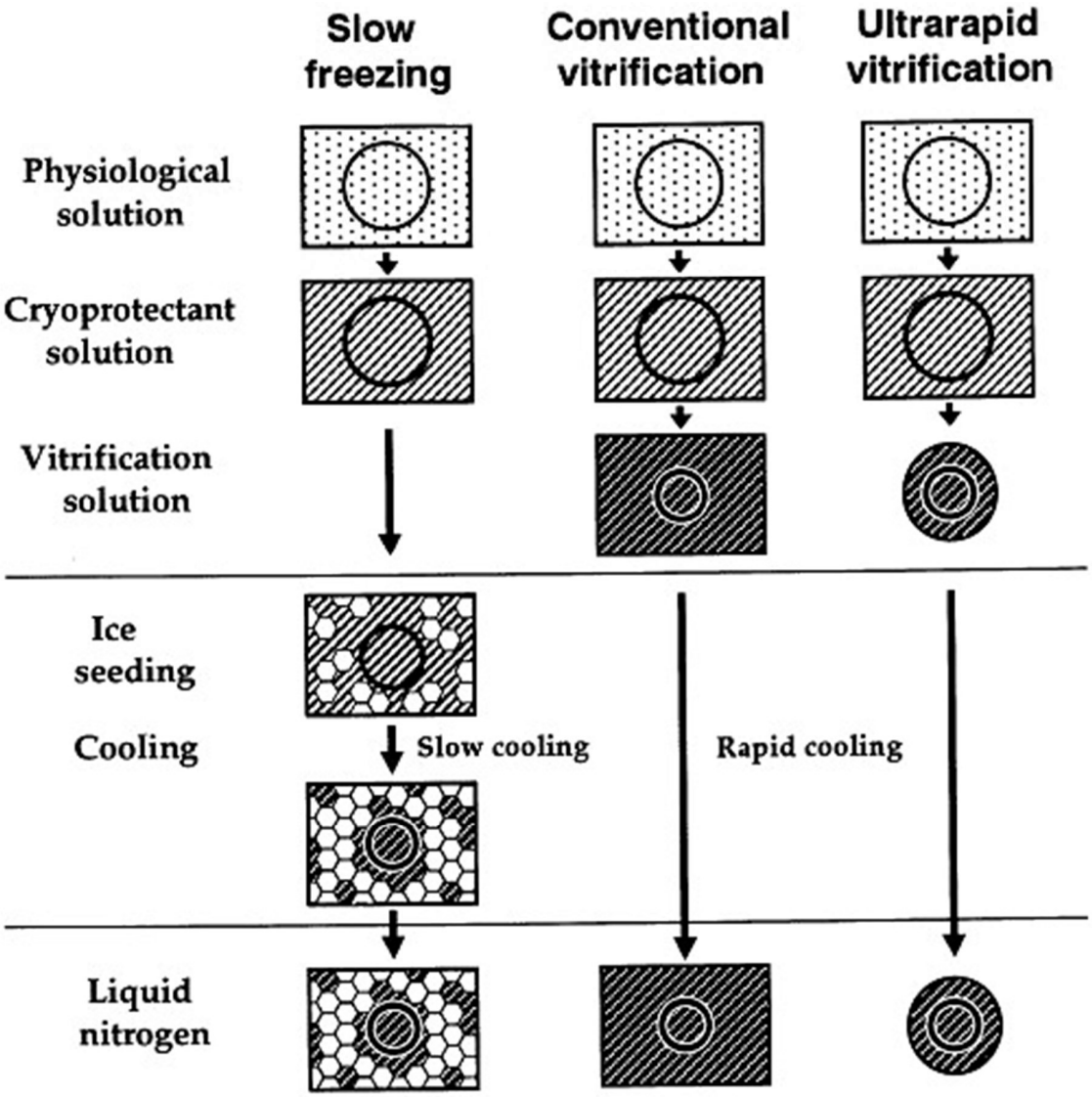

Figure 2.2 Schematic representation of an embryo during slow freezing and vitrification steps. Darker shading represents a higher osmolality. Embryo size changes represent shrinking and re-expansion in response to solution osmolality and cryoprotectant concentrations. Hexagons show ice crystal formation (Ferré et al., 2020).

Cryotop is the device for vitrification. The design facilitates for loading of the oocytes and makes it easy for vitrification (Kitasato, Japan). The Cryotop method represents a significant advancement in cryopreservation techniques, offering improved outcomes for individuals seeking fertility preservation and enhancing the success rates of assisted reproductive technologies. Its ability to rapidly vitrify and subsequently thaw cells with minimal damage makes it a cornerstone technique in modern reproductive medicine. So, cryotop become the gold standard for vitrification of animals such as rabbits (Hochi et al., 2004), bovine (Sprícigo et al., 2017), pigs (Galeati et al., 2011) and humans (Kuwayama et al., 2005).

Figure 2.3 Cryotop used for vitrification.

Vitrification is the process of solidifying a highly viscous liquid (Shaw and Jones, 2003). This method, known as a physical process, involves rapidly transforming high-concentration CPAs (cryoprotective agents) into a glass-like state at low temperatures (-196°C) (Hunt, 2019). Therefore, transforming a viscous liquid into a glass-like state helps reduce damage caused by ice crystal formation during the cooling process (Mahato et al., 2019). Vitrification technique can be separated into 2 parts including vitrified and warming/thawing parts. In vitrified step, there are 2 main factors: water and CPAs influencing cell survival rate (Leonard et al., 2003). Role of CPAs is to help decrease the freezing point and reduce ice crystal formation (Liu et al., 2021). However, addition of CPAs in solvent has been significantly influenced by increased osmolarity (Raju et al., 2021), which makes unstable in membrane polar absorption of oocytes. Consequently, These affect change of water in cells (Nolan and Hammerstedt, 1997). In the following step, warming/thawing is the process causing a rapid temperature to change and decrease formation of ice crystals. This process is involved in water influx into the cells of oocytes (Mphaphathi et al., 2023). Transfer of oocyte and embryo from high concentration of CPAs to isotonic solution causes a reverse osmotic shock or over-swelling. This phenomenon can be prevented by thawing oocyte and embryo in hypotonic solution (Fahy and Wowk, 2015). Monosaccharides and disaccharides such as sucrose are the most common CPAs

used in a thawing vitrification medium (Rajan and Matsumura, 2018; Sánchez et al., 2011) due to their ability to reduce osmotic stress, and prevent cell damage (Jin and Mazur, 2015; Pedro et al., 1997).

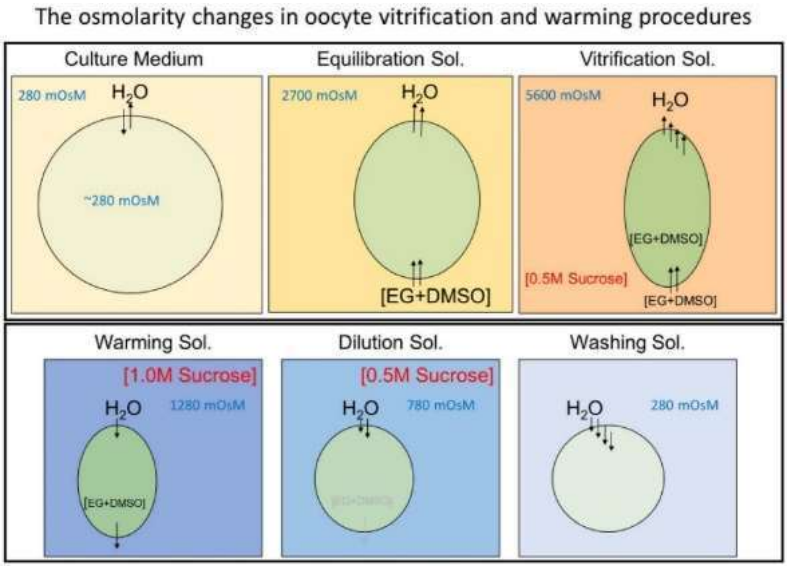

Figure 2.4 The process of vitrification and warming (Chang et al., 2022)**.**

2.6 Bovine blastocyst vitrification

Embryo cryopreservation through vitrification has been safely practiced since 1990. In 1990, vitrification was first successfully employed to cryopreserve cleavage-stage blastocyst of humans, leading to a viable birth (Gordts et al., 1990). As techniques of *in vitro* advance and the demand for single embryo transfer grows, there is a greater need for an efficient and dependable cryopreservation technique for surplus embryos. Cryopreservation was performed on zygotes immediately at the pronuclear stage after fertilization, embryos due to cleavage stages (2–8 cells), or at the expanded blastocyst stage. Vitrification of early-stage blastocysts is considered a preferable and superior alternative to a slow freezing rate due to its higher viability rate and improved pregnancy rates. Previous research has indicated that vitrified blastocysts using the open-pulled straw method exhibited developmental competence like that of fresh embryos. This suggests that the early blastocyst stage is the most optimal stage for the vitrification of bovine embryos (Martinez & Moreno, 2000). However, according to Michal et al. (2016), cryopreserving cleaved embryos are not as efficient as vitrifying blastocysts (Michal et al., 2016). Embryos, the highest quality have surpassed the developmental barrier linked to

genomic activation, and they exhibit relatively strong developmental potential. The blastocyst stage represents the last developmental phase before implantation, which offers the prime opportunity to screen and select the healthiest blastocysts for transfer (Ling et al., 2009). Additionally, the presence of numerous small cells provides an advantage, as the potential cell loss during the freezing and thawing process is likely to be less detrimental to the embryo's future development (Hunt, 2019). Moreover, during prolonged cultivation, embryos with poor survival are ceased in their result of development in no embryo cryopreservation needed (Wong et al., 2014). Hence, the vitrification of blastocyst-stage embryos plays a crucial role in assisted reproduction.

The optimal procedure for vitrifying mammalian embryos varies across different stages of development, even within the same species (Kasai and Edashige, 2010; Ling et al., 2009). The observed specificity may arise from variations in the cryobiological characteristics of embryos across different stages of development and species. These properties include sensitivity to cold temperatures, susceptibility to the potentially harmful effects of cryoprotectant chemicals, the ability of the cell membrane to allow water to pass through and cryoprotectants, and the ability to withstand changes in osmotic pressure causing swelling or shrinkage (Mazur, 2004). Likewise, The ability of embryos' plasma membrane to allow water and cryoprotectants to pass through remains crucial as a crucial property, as it is intimately linked to primary reasons for cell damage during vitrification (Edashige, 2017). It is essential to prevent such damage to ensure the viability of embryos after vitrification. As a result, the ability of the cell membrane to allow substances to pass through greatly influences the suitability of situations for vitrification.

Identifying the mechanism is essential for determining the optimal conditions for blastocyst vitrification. Determining the primary pathway for water movement and cryopreserved oocytes and embryos at various stages would aid in selecting appropriate cryoprotectants and optimal vitrification conditions. This includes various factors such as the duration, temperature, and steps involved in treating oocytes and embryos with cryoprotectant solutions before cryopreserved, as well as the process of removing cryoprotectants after warming (Prentice and Anzar, 2011). To develop protocols for vitrifying mammalian embryos, it's crucial to assess how water and cryoprotectants move at each developmental stage. During vitrification, the duration and temperature of exposure to the vitrification solution are critical factors due to its high cryoprotectant

concentration, which can be highly toxic to oocytes and embryos. The temperature and duration of exposure to vitrification solution are critical factors when cryoprotectants and water permeate embryos mainly via basic diffusion. This is because temperature directly influences the ability of water and cryoprotectants to pass through. When water and cryoprotectants primarily move through blastocysts via promote diffusion channels, the duration of showing to vitrification solution becomes more critical, since the permeability is less influenced by temperature. Nevertheless, subjecting blastocysts to vitrification solution at elevated temperatures could be modified, as cryoprotectants exhibit increased toxicity under such conditions

2.6.1 Vitrification and Warming solution

Today, many researchers are exploring various ways to enhance the success of cryopreservation by adjusting key factors such as cryoprotectants, freezing and warming rates, and adding chemical supplements like antioxidants. In a study by Chian et al. (2004), bovine embryos were first equilibrated in a solution containing 7.5% EG and 7.5% DMSO for 10 minutes. They were then transferred to a vitrification solution containing 15% EG, 15% DMSO, and 0.5 M sucrose for 30-60 seconds at room temperature before being loaded onto cryotop and plunged into liquid nitrogen. The warming procedure involved a stepwise dilution with sucrose solutions at 37°C, resulting in high survival and developmental rates post-thaw (Dujíčková et al., 2021). A similar study on bovine embryos utilized a vitrification protocol with a first solution containing 7.5% EG and 7.5% DMSO for equilibration, followed by a vitrification solution containing 16.5% EG, 16.5% DMSO, and 0.5 M sucrose. The embryos were placed on a cryotop and quickly plunged into liquid nitrogen. The warming procedure involved stepwise dilution with sucrose solutions of decreasing concentration at 37°C. This method resulted in improved survival and developmental rates for bovine embryos (Gómez et al., 2022). Interestingly, Hosseini and colleagues (2010) demonstrated a beneficial impact of antioxidants in the cultivation medium following embryo thawing. Likewise, the addition of **α-**tocopherol to the recovery culture medium significantly increased the blastocyst yield from post-warm bovine oocytes compared to control oocytes (Hosseini and Hosseini, 2017). Therefore, refining vitrification procedures with post-vitrification treatments could mitigate embryo sensitivity to cryopreservation, offering insights into advanced thawing procedures. Additionally, strategic selection of embryonic culture media is crucial for optimizing the freezing and warming processes successfully.

2.7 Oxidative stress by ROS and apoptosis

Oxidative stress is a monstrosity resulting from an imbalance between the generation and buildup of ROS in tissue and cells. ROS are small molecules. It acts as an oxidizing agent and is highly reactive. ROS is generated during the oxygen reduction step. These are called free radicals derived from oxygen, such as superoxide anions (O_2^-), hydroxyl radicals (OH) or non-oxidative molecules and hydrogen peroxide (H_2O_2) (Pizzino et al., 2017). In the IVP step, oocytes and embryos can be declarative to high levels of ROS because the antioxidant defense mechanism is lost and ultimately causing oxidative stress conditions (Agarwal et al., 2006). However, ROS can be produced within mitochondria as well as from external sources when cells are exposed to changes in environment culture factors, such as oxygen levels, light exposure, pH, and temperature (Caparros et al., 2021).

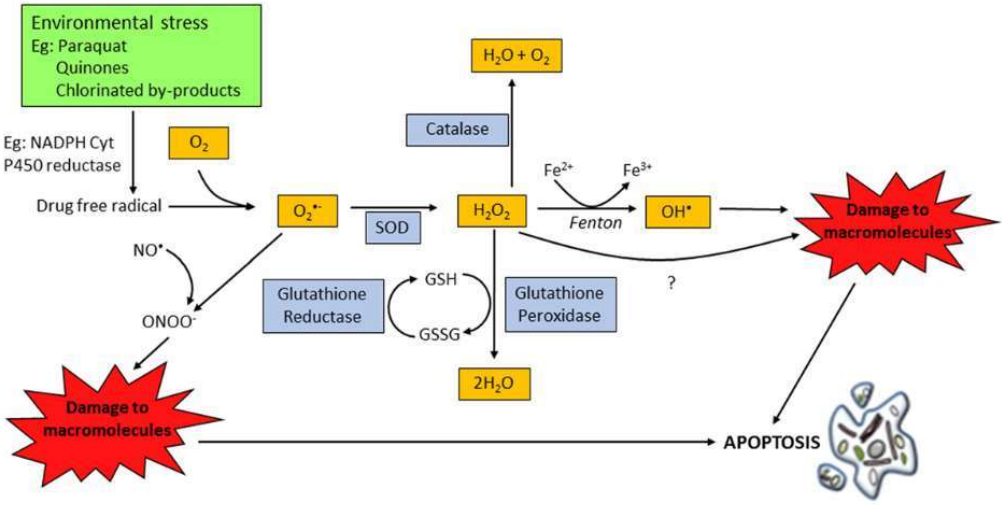

Figure 2.5 The production of ROS causes cell damage and apoptosis. Xenobiotics can contribute to the generation of xenobiotic-derived free radicals through one-electron reduction catalyzed by cytochrome P450 reductases (Kehrer and Klotz, 2015). The xenobiotic-derived free radicals rapidly react with oxygen to produce superoxide (O_2^-), which is reacted with nitric oxide (NO) to produce peroxynitrite ($ONOO^-$), or undergoes transformation to form H_2O_2, which is catalyzed by SOD. H_2O_2 can be detoxified by antioxidants such as catalase and GSH (Dutordoir and Bates, 2016). Alternatively, OH can be generated by metal-accelerated fenton reactions OH and $ONOO^-$ can damage intracellular proteins, lipids and nucleic acids. This causes to cell death by apoptosis (Vadgama, 2021).

Apoptosis, also known as the program of cell death, is a meticulously formulated and evolutionarily preserved process in which cells undergo self-destruction (Naessens, 2012). In multicellular organisms, it serves as a vital process for eliminating undesired or surplus cells due to the development or neutralizing of potential cells with DNA damage (Dutordoir and Bates, 2016). The maintenance of normal cellular balance depends greatly on the regulation of apoptosis. Apoptosis can be initiated by a range of external and internal signals, including various stressors like heat shock, viral infection, serum deprivation, hypoxia, DNA-damaging agents (such as radiation), and ROS (Pallepati, 2012). Multiple studies have affirmed that apoptosis plays a role in the reduction of oocyte and embryo development following vitrification (Sudano et al., 2011) and showed that cryopreserving could lead to apoptosis in bovine blastocysts. They discovered that the incidence of apoptotic cells in bovine embryos rose following undesired. Inaba et al. (2016) also discovered that vitrification of cattle embryos could lead to increased apoptosis after thawing.

2.8 Resveratrol

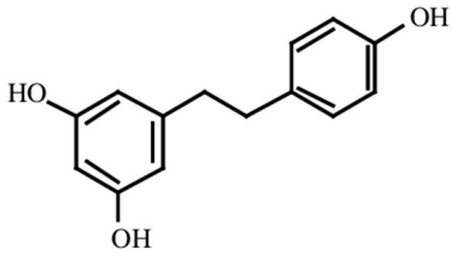

Figure 2.6 The chemical structure of resveratrol (3,5,4′-trihydroxy-trans-stilbene).

Resveratrol (3,5,40 -trihydroxytrans-stilbene) is a natural polyphenol antioxidant. It is found in many plants and foods such as peanuts, cocoa, grapes, and red wine. Its biological function is to shield the plant from parasitic attacks or environmental stresses, such as UV radiation and exposure to ozone (Dobrzynska, 2013). Resveratrol, a plant polyphenol, is synthesized when plants interact with microorganisms during pathogen attacks and is a protective agent against fungal and bacterial infections (Lattanzio et al., 2009). Resveratrol is present in cis- and trans-isomeric forms (Fig. 2.5). The trans-resveratrol form is steric and remains relatively stable when protected from high pH and light exposure (Lastra and Villegas, 2007).

Resveratrol exhibits various health benefits, including anti-inflammatory (Conte et al., 2015), antimicrobial (Pale et al., 2015), cancer prevention (Gusman et al., 2001), antioxidant (Zheng et al., 2012), heart protection (Tang et al., 2013), anti-cancer (Kucinska et al., 2014), and anti-aging (Smoliga et al., 2011) properties. For example, resveratrol facilitated bovine oocyte maturation and subsequent embryo development post-*in vitro* fertilization by stimulating progesterone secretion and exerting an antioxidant effect, likely through a mechanism dependent on sirtuin-1 (Wang et al., 2014).

Moreover, resveratrol has strong anti-apoptotic and antioxidant, serving as an antioxidative agent for IVP. The antioxidant potential of polyphenolic compounds depends on the redox properties of their phenolic hydroxyl groups and their ability to delocalize electrons throughout their chemical structure (Lastra and Villegas, 2007). Resveratrol has been found to operate through three distinct antioxidant mechanisms: (1) competing with coenzyme Q to reduce activity in the oxidative chain complex, where ROS is generated, (2) scavenging $O_2^{\bullet-}$ radicals produced in the mitochondria, and (3) inhibiting lipid peroxidation triggered by Fenton reaction products. Indeed, multiple studies have shown that resveratrol can scavenge both $O_2^{\bullet-}$ and $OH^{\bullet}$ radicals (Leonard et al., 2003; Martinez and Moreno, 2000). Many studies have demonstrated that resveratrol has a positive effect on the culture medium environment. The beneficial effects of resveratrol on reproductive system function and vitrification have been widely recognized in various species, including cats (Comizzoli et al., 2009), pigs (Lee et al., 2010), goats (Mukherjee et al., 2014) and cattle (Abe et al., 2017; Gaviria et al., 2019; Hayashi et al., 2019; Salzano et al., 2014; Sprícigo et al., 2017). The supplemental resveratrol can be categorized into phases: IVM medium, IVC medium, and vitrification or warming media.

Resveratrol treatment has been shown to create a beneficial microenvironment within the embryo increasing mitochondrial DNA copy number, GSH level and ATP content while decreasing ROS levels and downregulating apoptosis-related genes such as *Bax* and *Bcl-2* expressions. These effects can significantly enhance the blastocyst formation rate and quality after parthenogenetic activation or IVF (Abe et al., 2017; Hayashi et al., 2018; Wang et al., 2014; Wang et al., 2019). During IVC, treating cattle embryos with 0.5 μM resveratrol in the culture medium improves embryo quality, especially by increasing the total cell number in blastocysts (Gaviria et al., 2019). However, after cryopreservation, treating cattle embryos with 0.5 μM resveratrol resulted in similar numbers of inner cell

mass (ICM), trophectoderm (TE), and total cells (Salzano et al., 2014). Moreover, supplementing with the addition of resveratrol in the IVC medium before vitrification enhances embryo survival rates and cryotolerance. This is evidenced by higher development and hatching rates after post-warming culture, increased ATP generation in mitochondrial function through elevated Surtuin family member 1 (*SIRT1*) expression levels, and reduced lipid content (Abe et al., 2017; Salzano et al., 2014; Sprícigo et al., 2017). Interestingly, research has shown that the addition of resveratrol during the vitrification/warming procedure and in the IVC medium can have positive effects, which help embryos to partially restore the initial quality, compared to that of pre-cryopreserved embryos (Gaviria et al., 2019; Gaviria et al., 2019). The diverse impacts of resveratrol result from its ability to interact with numerous components of intracellular signaling pathways, influencing a broad range of enzymes such as kinases, lipo- and cyclooxygenases, sirtuins, and transcription factors (Frojdo et al., 2008)