Chapter

Importance of Supplementation during In Vitro Production of Livestock Animals

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Abstract

Increasing infertility is one of the most serious health problems of today. Over the past few years, we have had the opportunity to follow the progress of technologies focused on the production of embryos in vitro (i.e., in vitro fertilization and intracytoplasmic sperm injection, genetic engineering, or somatic cell nuclear transfer. Oocyte maturation is one of the most important processes in the production of embryos in vitro. Despite recent progress in this field, the developmental competence of in vitro generated oocytes is significantly lower than in vivo. In the last few years, a large number of studies dealing with the improvement of in vitro conditions for embryo culture have been published. These results have huge application potential in the reproduction of farm animals as well as in human medicine. Incorporating various elements, such as serum, hormones, growth factors, and antioxidants, can affect not only oocyte maturation or embryo culture but also an oocyte/embryo quality. The aim of this chapter is to summarize the most important types of supplementations of maturation and culture media and their impact on the improvement of in vitro oocyte and embryo production of farm animals.

Keywords: oocyte, embryo, in vitro embryo production, antioxidants, infertility

1. Introduction

In recent years, attention has been focused on the optimization and improvement of in vitro embryo production in the field of animal biotechnology and biomedical sciences. In vitro production of many high-quality embryos is a fundamental prerequisite for livestock production and development of further research areas (breeding, transgenesis, genetic engineering, xenotransplantation), as well as a way to address the issue of increasing infertility. Almost 30% of the worldwide countries use embryo technologies in the four most representative farm animal species—cattle, sheep, goats, and horses (reported in 2021). The number of In Vitro-Produced (IVP) embryos increased substantially in 2021 compared with 2020 for cattle, sheep, goats, and horses (**Table 1**) [1–3].

While the use of technologies in farm animals such as In Vitro Maturation (IVM), In Vitro Fertilization (IVF), and In Vitro Embryo Culture (IVC) is increasing, the

Year	Cattle	Sheep	Goats	Horses
2021	1,521,018	626	6355	11,619
2020	1,156,422	141	2275	8641
2019	1,031,567	1137	748	6303

Table 1.
Total production of IVP transferrable embryos.

transferable embryo yield is still very low. Currently, IVM is one of the most studied steps leading to increased success in embryo production not only in Assisted Reproductive Technology (ART) but also in biotechnology. IVM circumvents traditional superovulation methods, which require hormonal stimulation of females. Unfortunately, the pregnancy success rate using the IVM oocytes is lower than in vivo [4]. Suboptimal culture conditions during IVM can affect oocyte competence and the overall viability of the resulting embryo. Various culture conditions have been used across different species without realizing the need for optimization. Understanding critical aspects of the IVM technology can lead to the development of improved culture conditions, resulting in meiotically competent oocytes [5].

Optimized IVM is the first step leading to the production of competent embryos capable of producing viable offspring. Oocyte maturation is, therefore, a crucial step influencing the success of fertilization. However, its progress and completion are not easy to replicate in laboratory conditions.

There are two basic approaches to IVC. The first approach involves retrieving immature oocytes from antral follicles either from the ovary of slaughtered animals or transvaginally in living animals. Under optimal conditions, the oocyte will continue with meiotic division, ideally completing it within 18–48 hours depending on species and becoming ready for fertilization [6–8]. The second method, known as In Vitro Ovarian Follicle Maturation (IVFM), involves the culture of primordial or preantral follicles in vitro, inducing conditions that allow for the growth of different cellular types within the follicle [9]. The success of follicle cultivation in livestock animals is very low and limited only to secondary preantral follicles [9, 10].

In addition to the quality of biological material and the experience of the manipulator, the success of IVP is also influenced by the in vitro culture environment. During in vitro/in vivo maturation, the oocyte undergoes physiological maturation, which is characterized as a complex biological process of transition between diakinesis and metaphase I of meiosis. The transition is accompanied by several changes at the level of the nucleus, cytoplasm, and epigenetic changes, which determine the ability of oocyte to achieve developmental competence. This competence is characterized as the ability of the oocyte to complete preimplantation development by reaching the blastocyst stage, subsequently initiating and maintaining gravidity, eventually resulting in the birth of a healthy offspring [10–12].

However, the success of the IVP technique is often limited due to various factors such as poor oocyte quality, inadequate oocyte maturation and embryo culture conditions, and suboptimal embryo development. To overcome these problems, researchers have investigated the use of various supplements to improve the quality and quantity of mammalian embryos produced in vitro.

This chapter aims to summarize the recent progress in IVP embryo production, particularly in two steps: oocyte maturation and embryo culture with an emphasis on the main livestock animals.

2. Oocyte maturation and embryo development

2.1 In vivo environment

Acquiring developmental competence is a strictly regulated process that is very susceptible to errors. This fact can have serious consequences for fertility and, under certain circumstances, also for the health of the offspring [13]. Meiotic division in oocytes is initiated based on hormonal stimuli and regulated via multiple signaling pathways. Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) initiate ovarian folliculogenesis and the resumption of meiosis, resp. The process of meiosis involves the Breakdown of Germinal Vesicle (GVBD), formation of the spindle during the first Metaphase (MI), segregation of homologous chromosomes, extrusion of the first Polar Body (PBI), and arrest of division at the metaphase of the second Meiotic division (MII).

LH binds itself to the LHR receptor present in the granulosa cells, leading to the activation of a whole series of oocyte and follicular changes. The LH/LHR induces activation of cyclic adenosine monophosphate/protein kinase A (cAMP/PKA) in mural granulosa cells, and Epidermal Growth Factor (EGF) and EGF-like factors production and secretion. The binding of EGF to its receptor (EGFR) in cumulus cells (CCs), leads to Mitogen-Activated Protein Kinase 3/1 (MAPK3/1) (Extracellular Signal-Regulated Kinase 1/2 (ERK1/2)) activation. Moreover, EGFR signaling elevates calcium in CCs, dephosphorylates and inactivates Natriuretic Peptide Receptor B (NPR2), and decreases Cyclic Guanosine Monophosphate (cGMP) levels [14]. Communication between the oocyte and its surrounding CCs is a prerequisite for the oocyte to acquire efficient developmental capacity [15]. LH induces the phosphorylation of connexin 43 (GJA1) and connexin 37 (CX37 or GJA4), which are the main components of gap junctions between CCs and cumulus cells-oocytes, resp. CX37 is essential for oocyte-cumulus cells communication because it forms intercellular membrane channels of gap junctions and thereby allows inorganic ions and small molecules to share and is essential for follicle growth [16]. Phosphorylation of connexins leads to the closure of gap junctions and disruption of cGMP levels in both CCs and the oocyte. Decreasing levels of cGMP in oocytes lead to the removal of inhibition of the hydrolytic activity of Phosphodiesterase 3A (PDE3A), resulting in a decrease in (cAMP). The absence of sufficient cAMP level in the oocyte stops the PKA pathway. Thus, the inhibitory Phosphorylation of Cyclin-Dependent Kinase1 (CDK1) at tyrosine 14 and threonine 15 caused by Wee1-like protein kinase 2/ Myelin transcription factor 1 (WEE1B/MYT1) is removed by G1/S-promoting phosphatase (CDC25) activated by PKA. Removing inhibition allows cyclin B to enter the nucleus, where it forms an active Maturation Promoting Factor (MPF) with Cyclin Dependent Kinase 1 (CDK1). The MPF is a serine/threonine kinase that is required for meiosis resumption [17].

Both nuclear and cytoplasmic maturation of the oocyte and early embryo development before compaction rely on ATP-provided energy. Mitochondria are the main powerhouses that generate ATP through Oxidative Phosphorylation (OXPHOS) [18]. As a by-product of OXPHOS system, free radicals are formed. These Reactive Oxygen Species (ROS) are naturally balanced by endogenous antioxidants keeping their concentration at levels which is beneficial for oocytes or embryos [19].

2.2 In vitro culture

Once retrieved from follicles, the oocytes suffer from the lack of a supporting environment. Also, embryos cultured in vitro are exposed to quite unnatural

conditions. To mimic *in vivo* conditions, it is important to consider not only the level of CO_2/O_2, temperature, pH, humidity, and manipulation speed, but also the composition of the maturation medium. The maturation medium is supplemented with several species-specific components such as hormones (HCG, PMSG, FSH, LH, melatonin, etc.), Growth Factors (GFs) as Insulin-like Growth Factor-1 (IGF1), Fibroblast Growth Factor 2 (FGF2), Leukemia Inhibitor Factor (LIF), EGF, serum or its component as Bovine Serum Albumin (BSA), Fetal Bovine Serum (FBS), Human Serum Albumin (HSA) [5, 6, 20–22]. The composition of maturation/culture media can affect gene expression, gene methylation, level of mRNA, and other important cell processes in both oocyte/embryo and the born offspring [23–25].

The mechanisms leading to the resumption of meiosis in vitro are different from those in vivo [26]. Cumulus cells lack the responsivity to LH because they either lack LHR or express them at an insufficient level. To enhance developmental competence of oocytes, pre-culture systems with chemical compounds that delay GVBD or enable LHR formation and internalization into cell membranes were also tested. Inhibitors of MPF, such as cycloheximide, roscovitine, or butyrolactone [27], enhancers of cAMP levels in cumulus cells (osobutylmethyl xanthine (IBMX) [28] or its analog dibutyryl cyclic Adenosine Monophosphate (dbcAMP), [29] or cAMP or PDE3A inhibitor [30] add to media can postpone meiotic resumption and improve developmental competence.

Among the most used serums or their alternatives are FBS, Fetal Calf Serum (FCS), estrus goat or sheep serum, BSA, polyvinylalcohol or polyvinylpyrrolidone. BSA is a protein that provides the necessary amino acids and nutrients for the growth and development of embryos. It also helps to stabilize the pH of the culture medium, which is important for maintaining a suitable environment for embryonic development. Serums contain growth factors (GFs) and hormones that promote cell growth and division. They also provide essential nutrients such as vitamins, minerals, and lipids needed for embryonic development. However, it is important to note that the quality of serums may vary depending on the source and processing methods. Moreover, using these components lacks the reproducibility. To ensure optimal results, careful selection of high-quality sources of these supplements is important [31–33]. The main negative of using Follicular Fluid (FF) or FBS is the potential contamination of the medium. In the case of contamination, the pH and osmolarity of the medium change, which can affect the viability and metabolism of the oocytes. In addition, the FF contains various proteases and hydrolases that can degrade the extracellular matrix surrounding the oocyte, leading to premature oocyte activation and degeneration [34]. Thus, researchers are making an effort to withdraw these components and switch to chemically-defined or at least semi-defined media [20]. Chemically-defined media contains a precisely known combination of nutrients and growth factors. Semi-defined media fall somewhere in between undefined media, which refers to a mixture of various components and defined media, so it contains some known components but also includes undefined substances. For successful IVP and embryogenesis research, defined and semi-defined media allow more precise control over the environment and nutrients provided to the embryos [20, 35]. Lipid content in oocytes is species-specific with the highest content in porcine oocytes [36]. Thus, cumulus cells surrounding oocytes must provide fatty acids for ATP synthesis in oocytes of other species. Co-culture of oocytes with oviductal epithelial cells, ovarian cortical cells, fibroblasts, cumulus cells isolated from matured follicles, etc. increases the number of matured oocytes and improves their quality. The cumulus cells provide essential nutrients and growth factors that help the oocyte mature properly. In addition, co-culture of oocytes with cumulus cells has been shown to

increase the fertilization and embryo development rates (77.2% of matured oocytes after co-culture with cumulus cells versus 62.1% after culture in conventional way in human oocyte [37, 38].

Oocyte maturation and first embryo divisions are dependent on a relatively low energy supply, the majority of which is generated by endogenous OXPHOS system from pyruvate or fatty acids [39]. As development proceeds, the embryo starts to utilize glucose as a high energy source [36]. Moreover, the developing embryo must overcome a species-specific likelihood of developmental block which correlates with the cytoplasmic quality of the oocyte. In case the embryo does not activate its genome, it cannot advance in successful development and arrests.

In vitro-produced oocytes and embryos are exposed to conditions that can induce elevated levels of ROS leading to mitochondria damage, the oxidation of specific amino acids, and lipid peroxidation, ultimately inducing cellular damage, apoptosis (programmed cell death), and the loss of appropriate ATP supply [19]. To minimize or eliminate the detrimental biological effects of ROS, different exogenous antioxidants or fatty acid oxidation modulators added to the media were tested [40]. Antioxidants are generally known to decrease ROS levels [41].

In the following chapters we will therefore focus on the review of media supplementation used in embryo IVP of farm animals (**Table 2**).

Substitution	Animal	Benefit	Reference
Hormones, gfs, serum			
FBS	Pig	Improved fertilization rates and embryo development	[28, 42]
	Cattle	Higher blastocyst rate	
	Horse	Sperm capacitation	[43]
ECS	Cattle	Promoting cell proliferation and embryonic development	[44]
EGF	Pig	Improved fertilization rates and embryo development	[28, 42]
	Horse	Improved oocyte maturation	[45]
IGF1	Pig	Improved fertilization rates and embryo development	[28, 42]
	Cattle	Improved embryonic development	[46]
LIF	Pig	Improved fertilization rates and embryo development	[28, 42]
	Cattle	Improved embryonic development	[46]
	Sheep	Fertilization rate, blastocyst quality	[47]
	Goat	Maturation rate	[48]
FGF2	Pig	Improved fertilization rates and embryo development	[28, 42]
	Cattle	Improved embryonic development	[49]
	Sheep	Oocyte maturation	[50]
LH	Pig	Improved oocyte maturation	[51]
FSH	Pig	Improved oocyte maturation	[51]

Substitution	Animal	Benefit	Reference
Melatonin	Pig	Improved oocyte maturation, fertilization rate, embryo quality	[52–55]
	Cattle	Improved maturation rate, blastocysts rates, embryo quality	[56–59]
	Sheep	Improved maturation rate, embryonic cleavage	[60–62]
	Goat	Improved oocyte maturation, higher-quality blastocysts	[63]
Hyaluronic acid	Cattle	Oocyte maturation, embryo competence, and quality	[64]
Cysteamine	Cattle	Improved blastocyst rate	[65]
	Goat	Improved maturation rate (in combination with LIF and Y27362)	[48]
Antioxidants			
Vitamin E, vitamin C	Pig	Enhance lipid metabolism, improved embryo rates, and quality	[66]
Niacin (vit B3)	Pig	Increased blastocyst rate	[67]
L-carnitine	Pig	Improved embryo quality	[68]
	Cattle	Improved embryonic development, increased blastocyst number	[41, 69, 70]
	Sheep	Improved blastocyst rates, Reduced ROS level	[71]
Resveratrol	Pig	Improved oocyte and embryo quality	[72, 73]
	Sheep	Improved blastocyst rates	[74]
	Goat	Improved blastocyst rates in prepubertal goats	[75]
Astaxanthin	Pig	Improved quality of embryos, promote development of blastocysts	[76, 77]
Quercetin	Cattle	Increased blastocyst number	[41]
Folic acid	Cattle	Improved DNA methylation and blastocyst development	[78]
Lycopene	Cattle	Improved oocyte maturation, improved embryo quality	[79–82]
DMSO	Cattle	Increased first polar body extrusion, higher number of blastomeres in blastocysts	[82]
Sericin	Sheep	Increased maturation and CC expansion	[83, 84]
GSH	Cattle	Improved IVP	[85, 86]
	Sheep	Improved developmental competence Competence	[87]
Royal jelly	Goat	Higher blastocyst rate, decreased apoptosis	[88]
Linolenic acid	Goat	Higher blastocyst rate	[89]
L-ergothioneine	Cattle	Higher number of cells in ICM	[70]
	Sheep	Higher blastocyst rate, Reduced ROS level	[90]
L-ascorbic acid (in combination with ITS)	Cattle	Improved amount and quality of embryos	[91]

Table 2.
Benefits of the media supplementation.

3. Pigs

Pigs represent one of the most suitable model organisms for study of mammalian preimplantation development with potential applications in human medicine. Based on their similar anatomy, physiology, and genome, pigs are used in research of genetic diseases, biotechnology, gene engineering, xenotransplantation, and embryotechnology [92, 93].

Although the first in vitro-produced porcine embryo was reported in 1986, [94] the technology in this species has the lowest success rate compared to other model organisms. This is caused, in particular, by insufficient synchronization of nuclear and cytoplasmic maturation and high rates of polyspermy [28]. Porcine oocytes and embryos contain more lipids than other mammals which made them more frangible after vitrification [36]. On the other hand, this should be beneficial for pig embryos, because they have decreased metabolism of pyruvate in comparison to other ruminant's embryos and they rely on other energy sources, such as glucose or lipids. Therefore, the attention of experts is focused on modifying in vitro protocols to increase the success of IVP. One of the basic parameters is the supplementation of maturation and culture media.

3.1 Serum, hormones, and growth factors

FBS is a commonly used supplement in the culture medium for oocyte growth and maturation in pigs. But as mentioned earlier, FBS can contain various pathogens such as viruses, bacteria, fungi, prion proteins, and mycoplasmas that can infect and damage oocytes and embryos. In addition, FBS may also contain endotoxins, which can induce inflammatory reactions in oocytes and reduce their viability and quality [51].

GFs are proteins that play an important role in cell growth and differentiation. In the embryo production, they can help support maturation of oocytes and improve their quality. Adding these factors as an EGF, IGF1, LIF, and FGF2, especially in combination with serum alternatives, into the maturation medium can improve fertilization rates and embryo development [42, 95]. However, it is important to note that the optimal concentration and combination of growth factors may vary depending on the specific needs of each experiment. Therefore, careful consideration should be given to the selection of GFs to be included in the maturation media for successful IVP.

Hormones play a crucial role in the maturation media used for IVP in pigs. FSH and LH are commonly included in the maturation media to support oocyte maturation. In addition, other hormones such as estradiol and progesterone can also be added to the maturation media. Estradiol promotes follicular development and helps regulate the estrous cycle, while progesterone prepares the uterus for the implantation of a fertilized egg [96, 97].

Melatonin is a hormone that is naturally produced by the pineal gland in animals, including pigs. It plays a crucial role in regulating the sleep-wake cycle and is supposed to be a potent antioxidant, anti-apoptosis, and anti-inflammatory agent. Melatonin is a binder of nitric oxide radicals, and one of its metabolites inhibits the activity of nitric oxide synthase, thereby affecting oxygen consumption in embryos [98, 99]. Melatonin improves oocyte maturation, fertilization rate, and embryo quality of IVP pigs [52]. Adding melatonin to the culture media during IVC can increase the rate of blastocyst formation and reduce levels of oxidative stress. When oocytes were matured in medium supplemented with melatonin, the embryo quality was

improved in the blastocyst formation (10.7 vs. 4.2%). Moreover, the proportion of fragmented DNA nuclei in blastocysts was reduced (2.1 vs. 7.2%) [53]. Additionally, melatonin supplementation can improve the cryotolerance of pig embryos [54]. In contrast, in [55] melatonin supplementation during IVM did not significantly improve the rate of pig oocyte maturation or blastocyst formation after parthenogenetic activation. On the other hand, melatonin treatment during IVC reduced ROS levels in parthenogenetic embryos. Overall, adding melatonin as a supplement during IVP of pig embryos shows promising results for improving embryo quality and increasing success rates. The inclusion of hormones in the maturation medium can significantly improve the success rate of in vitro embryo production in pigs. However, it is important to carefully balance the concentrations of hormones to avoid negative effects on oocyte quality or embryonic development.

3.2 Antioxidants

One of the differences between pigs and the other species is the amount of lipids. Lipid metabolism plays a crucial role in determining the developmental competency of pig oocytes and embryos [100]. Compounds known as antioxidants can neutralize free radicals and diminishing oxidative stress. Research has illustrated that including antioxidants like Glutathione (GSH), vitamin E, and vitamin C (ascorbic acid) in the culture medium can enhance lipid metabolism and optimize embryo quality during in vitro fertilization [101]. Furthermore, these particular antioxidants reduce the occurrence of lipid peroxidation while promoting optimal embryonic development throughout IVP.

The addition of niacin (vitamin B3) to culture media during porcine increases blastocyst formation rates, reduced apoptosis, and enhances cell proliferation. Furthermore, niacin improves mitochondrial function critical for energy production during embryonic development [67]. Several antioxidants are effective in improving the quality of in vitro-produced pig embryos. Vitamin E improves embryo development rate and reduces the incidence of abnormalities. Similarly, vitamin C and B3 improves embryo quality and increases the rate of blastocyst formation. L-carnitine (L-car), a naturally occurring amino acid, plays a key role in energy metabolism and free radical neutralization [69]. Supplementation with L-car improves the quality and viability of in vitro-produced pig embryos. L-car acts as a carrier molecule for fatty acids, which are the primary source of energy for oocytes and early developing embryos. Adding L-car to the culture medium during IVP can significantly increase the blastocyst formation rate and improve overall embryo quality [66, 68, 102]. This improvement occurs because L-car enhances mitochondrial function, leading to increased ATP production and improved cellular metabolism. Moreover, L-car improves lipid metabolism in porcine oocyte [103].

Resveratrol, a naturally occurring polyphenolic compound, has numerous health benefits, including anti-inflammatory, antioxidant, and anti-cancer properties. In porcine embryo production, resveratrol improves the quality of oocytes and embryos. Resveratrol supplementation during IVM can enhance their developmental competence. This is attributed to the ability of resveratrol to reduce oxidative stress and improve mitochondrial function in oocytes. Furthermore, resveratrol enhances the developmental competence of porcine embryos during IVC. This is achieved through the activation of Sirtuin 1 (SIRT1), a protein that plays a crucial role in regulating cellular processes such as metabolism, DNA repair, and stress response. Resveratrol activates SIRT1, which in turn promotes the development of porcine embryos by improving their quality and reducing apoptosis [72, 73].

Astaxanthin, like L-car, is a naturally occurring carotenoid able to capture free radicals and protect cells from oxidative damage. It is found in various seafood and is used as a dietary supplement for its antioxidant properties. Its natural origin makes it a safe alternative to synthetic antioxidants commonly used in laboratory conditions. Studies have shown that supplementing with astaxanthin can improve the quality of in vitro-produced pig embryos by reducing oxidative stress and increasing cell proliferation. It has also been found to promote the development of blastocysts, which are key to successful embryo implantation [76, 77].

4. Cattle

IVP in cattle serves as a valuable tool to propagate genetically superior animals and to obtain faster more embryos [104]. Together with pigs, cattle are the most used model organism to study preimplantation development in mammals. This is based not only on the similarity of its preimplantation development with humans and other mammals but also on the relative ease of bovine embryo culture. The world's first calves obtained exclusively by IVP, that is, IVM of oocytes, IVF as well as IVC, were reported in the late 1980s [105].

Nevertheless, notable inefficiencies exist in IVP embryos, where only 20 to 40% of oocytes produce transferable embryos and only 30 to 40% of transferred IVP embryos result in calves [85, 106]. The composition of culture media for embryos tries to mimic the natural environment of the uterus and oviduct. Several studies have demonstrated the effect of adding different embryokines to the culture medium on bovine blastocyst cell numbers and post-transfer pregnancy outcomes, but the results are not uniform [107]. Nevertheless, the optimal composition of culture media has not been established.

4.1 Serum, hormones, and growth factors

The culture media are commonly supplemented with FBS or BSA as a source of proteins for the developing embryo. Nevertheless, only a few studies show a beneficial effect of FBS supplementation. When culture is performed in the presence of FBS, the blastocyst rate is higher than in BSA . On the contrary, FBS supplementation stimulates higher apoptosis occurrence, lower Inner Cell Mass/Trophectoderm (ICM/TE) ratio, reduces proteosynthesis or alters the expression of genes important for early embryogenesis [20]. Indeed, decreased serum concentration in bovine embryo culture improves the expanded blastocysts rate, similar to BSA [108]. Supplementation of Estrous Cow Serum (ECS) to culture media during bovine oocyte or embryo culture promotes cell proliferation and preimplantation embryo development by reducing endoplasmic reticulum stress. ECS supplementation can also decrease macroautophagy to promote advanced embryo development [44]. As suitable replacement for FBS can be used FGF2, which has a positive effect on bovine embryonic development [20, 46, 49], especially in combination with other growth factors like LIF, IGF1, or Transforming Growth Factor β1 (TGF-β1) [109]. Similarly, recombinant Low Molecular Weight Human Fibroblast Growth Factor 2 (recLMW-hFGF2) supplementation increases oocyte maturation, embryo yield, and developmental competence [110]. Hyaluronic Acid (HA) supplementation has improved bovine oocyte maturation, embryo quality, and their developmental competence. In addition, HA also reduced apoptotic index and ROS [24, 64].

In addition to growth hormones, sera, etc., some less common supplements can be added to culture media. For example, supplementation of CX37 led to a significantly higher portion of cleavage embryos and the number of obtained blastocyst [111].

GSH is an essential antioxidant for oocyte maturation, fertilization, and early development [85]. GSH is synthesized from three amino acids—cysteine, glycine, and glutamate (CAA). Intracellular GSH level in zygotes during fertilization in a medium supplemented with CAA was higher than in the control group. On the contrary, CAA supplementation negatively affects oocyte penetration and subsequent embryo development [112] probably due to their harmful effect on sperm. Higher concentrations of intracellular GSH improve IVP in cattle [65], buffalos [113], sheep [114], and pigs [115]. Indeed, GSH added to culture media significantly increased the blastocyst rate and embryo quality [86].

The best results of culture are obtained especially when the combination of the above-mentioned supplements is used. The supplementation with the combination of EGF, IGF1, and CX37 significantly improves oocyte maturation and embryonic development due to decreased ROS level, increased level of TZP (the transzonal projection), GSH, ATP-content, and mRNA expression level. The results are even better when also FSH and LH were added to the medium [111]. Similarly, the supplementation of Insulin, Transferrin, and Selenium (ITS) alone does not affect embryo production. However, the combination of ITS and L-ascorbic acid (AA) during the last 12 hours of oocyte maturation improved the amount and quality of bovine embryos [91].

Melatonin enhances oocyte maturation rate, promotes the expansion of CCs, and influences mitochondrial distribution patterns. Increased rates of dispersed mitochondria in the cytoplasm were observed in oocytes [56]. Moreover, ROS level significantly decreases after melatonin supplementation while GSH concentration significantly increases. Blastocyst rates after oocyte melatonin treatment were also significantly higher and the incidence of apoptotic nuclei in blastocysts decreases [57]. Melatonin improves oocyte maturation and embryo quality, especially through its antioxidative and anti-apoptotic effects and improving gene expression pattern [57–59].

4.2 Antioxidants

Quercetin, vitamin C, L-car, melatonin, or folic acid are examples of antioxidants with the potential to reduce ROS levels when supplemented in media. The maturation of bovine oocytes in the presence of these antioxidants leads to increased blastocyst number and improved quality (evaluated by total cell numbers), except for quercetin supplementation with unenhanced embryo quality [41]. Zygotes co-cultured with several doses of L-ergothioneine exert no significant effect on cleavage rates and blastocyst development. However, a significantly higher number of cells were found to be allocated to the inner cell mass at 8-day-old blastocysts [116]. L-cysteine improves oocyte nuclear maturation rate and CCs expansion.

Folic acid is an antioxidant naturally present in FF and its employment as a supplement of culture media thus offers itself. Folic acid addition to culture media improves DNA methylation of IGF2 gene and accelerates the development of blastocysts [78].

L-car supplementation leads to increased quality of less competent oocytes demonstrated by a significantly higher rate of mitochondrial clusters and significantly lower content of lipids in these oocytes. The supplementation also improves the normal fertilization rate of less competent oocytes [70]. L-car increases the mobilization of fatty acids from ooplasm into the mitochondria to facilitate β-oxidation of fatty acids [41].

Lycopene, an antioxidant found in tomatoes and other red fruit and vegetables, increases oocyte maturation rates. Moreover, it improves also the cleavage rate and development to the blastocyst at day 8 [79, 80] and reduces Total Apoptotic Cells/Total Cell Number (TA/TCN) ratio and intracellular ROS concentrations in these blastocysts. Lycopene supplementation in serum-free media decreases ROS production in matured oocytes and exerts the same effect as for the higher blastocyst yield and lower TA/TCN ratio [79, 81]. This antioxidant could not restore oocyte damage when pro-oxidant menadione was added to the maturation media [81]. Moreover, lycopene did not improve the blastocyst rate when oocytes have been matured under heat shock conditions [79].

Among others, the role of Dimethyl Sulfoxide (DMSO) supplementation during oocyte maturation and embryo development was explored, since the low doses of DMSO have also an antioxidant effect. The presence of 0.5% DMSO increases first polar body extrusion after IVM and leads to higher GSH and a higher number of blastomeres in the blastocysts [82].

5. Sheep and goats

The use of small ruminants (goats, sheep) as model organisms for studying preimplantation development is less common than the use of pigs and cattle. Sheep and goats have become important model animals in pharmaceutical and biomedical research due to their short gestation period and secretion of milk. Moreover, they are a suitable model organism for humans due to similarities in size, anatomy, and speed of preimplantation development. In sheep and goats, several genome engineering techniques can be applied. Purposes for these applications are the investigation of gene functions, production of disease-resistant or genetically superior animals, production of pharmaceuticals in milk, modeling of human diseases and xenotransplantation [117].

5.1 Serum, hormones, and growth factors

In [83], chemically-defined medium supplemented with FGF2, LIF, and IGF1 (FLI) and sericin during IVM significantly improves blastocyst rates [118]. Besides commonly used factors, of which FLI is composed, also some paracrine factors seem to be suitable supplementation for ovine oocyte maturation. Using Connective Tissue Growth Factor (CTGF), Stromal-Derived Factor 1 (SDF1), Nerve Growth Factor (NGF), and Hepatocyte Growth Factor (HGF) in defined concentrations improved polar body extrusion rate. Moreover, the combination of all of them promotes CCs expansion and enables even higher oocyte maturation. Enhancement of parthenogenetic activated embryos yield coincides with the higher blastocyst's cell number. Moreover, the apoptosis in oocytes, CCs, and embryos decreases [119]. Supplementation with these paracrine factors could also be a promising alternative for ovine IVF culture media improvement. IGF1 and EGF supplementation increases maturation and cleavage rates in ovine. Moreover, both GFs improve the percentages of cleavage, morula, and blastocyst obtained from more competent oocytes [120]. Ovine oocytes also positively respond to EGF supplementation into maturation media [121].

Similarly, the addition of melatonin in defined concentration also improves maturation rate and early embryonic cleavage, but unlike sericin, melatonin did not

improve blastocyst rate [60]. These results are surprising because melatonin is generally used for breeding season prolonging of ewes and it is presumed that, because of its positive effect on fertility rate and litter size, melatonin can affect embryo survival and improvement of embryo quality [61]. When the maturation medium for prepubertal lamb oocytes was supplemented with melatonin, there was no significant difference in maturation rate [83]. More information about melatonin´ influence on sheep oocytes in vivo and in vitro is summarized in a meta-analysis-based research article by [62]. Melatonin added to culture media significantly increased the percentage of MII oocytes and blastocysts rate and elevated levels of GSH [122]. Its supplementation can also improve oocyte maturation and increase the number of higher-quality blastocysts in juvenile goats [63].

FGF2, ITS, and LIF can also be included among the most commonly used supplements. FGF2 promotes oocyte maturation, fertilization, and embryonic development by stimulating granulosa cell proliferation and differentiation. ITS provides essential nutrients for cell growth, while LIF promotes embryo survival by preventing apoptosis and promoting pluripotency. The combination of these three supplements led also to increased fertilization, cleavage, and blastocyst rates [48]. Supplementation of maturation media with a combination of FGF2 and ITS led to significantly higher maturation rates than in the control group at sheep oocytes (82.43 vs. 63.83% in control group) [50]. On the other hand, LIF treatment significantly increased fertilization rate (established by the 2-pronuclear stage). Although, blastocyst rate was not increased in sheep or lamb embryos, the quality of originated blastocyst was significantly improved [47]. LIF has also a beneficial impact on the goat maturation rate. When a combination of LIF and cysteamine was used in the maturation medium, a significantly higher number of MII oocytes were observed (60 vs. 45% in control). Moreover, co-culture with these two substances and with Y27362 (inhibitor of the ras homolog gene family, member A (RhoA) and its downstream effector Rho-associated protein kinase ROCK (RhoA/ROCK) pathway important for cell proliferation and apoptosis) together demonstrated the highest maturation rate (67.5%) [48].

5.2 Antioxidants

Sericin is considered as one of the suitable antioxidants to enhance the growth and development of goats/sheep embryos. A suitable concentration of sericin increases maturation of oocyte and CCs expansion rate. Moreover, embryonic cleavage and morula and blastocyst development rates can be also significantly enhanced [84].

As in bovine oocytes, the effect of exogenous GSH supplementation was tested also in ovine oocytes. The addition of exogenous GSH to the maturation medium improved developmental competence, gene expression, and histone methylation, especially in oocytes matured from COCs with less number of cumulus cell layers [87].

An interesting alternative is the use of royal jelly. The supplementation of royal jelly to the maturation medium increases the maturation rate and enhances GSH content in goat oocytes. Royal jelly supplementation leads to a higher proportion of blastocysts and decreased number of apoptotic cells per blastocyst. Similarly, the expression of pro-apoptotic genes decreases. Authors presumed that the positive effect of royal jelly may be caused by various active compounds, i.e., proteins, essential amino acids, vitamins, lipids, and sugars [88]. Similar positive effects were reported also about linolenic acid supplementation in the maturation medium of goat [89].

In addition to resveratrol supplementation in porcine maturation and culture media, the usage of resveratrol has also been found to improve development of sheep and goat embryos. Resveratrol during IVM and IVC significantly improves morula and blastocyst rates and increases total cell number at blastocyst stage [74]. The supplementation of resveratrol to culture media of prepubertal goat oocytes leads to a significantly higher blastocyst rate. However, it does not enhance the blastocyst quality, mitochondrial activity, ROS level, and ATP content [75].

L-car supplementation has also a beneficial effect on sheep oocyte/embryo culture. Oocytes cultured with L-car achieved higher cleavage and higher morula and blastocyst development rates, however, maturation rate was not significantly improved. L-car treated oocytes and embryos that arose from oocytes co-cultured with L-car decrease the intracellular ROS levels and increase the GSH compared to untreated controls. Moreover, the addition of L-car to a medium containing hydrogen peroxide (H_2O_2) can protect oocytes and embryos from H_2O_2-induced oxidative stress (intact DNA preservation) proofing the antioxidant effect of L-car [71]. A positive effect on ROS levels has also L-ergothioneine supplemented to the maturation medium. After supplementation, intracellular ROS level is reduced and GSH level is increased in oocytes. Moreover, the antioxidant effect was also proven by the same experiment with H_2O_2 treatment with the same results where L-ergothioneine protected embryos from the impact of H_2O_2. The success of maturation was not elevated, but L-ergothioneine improved embryo cleavage and morula and blastocyst rates [90].

6. Horses

Compared to other livestock animals, efficiency of mare's IVF is very low—around 33% of fertilized oocytes. These relatively low success rates are probably due to the inability of spermatozoa to penetrate the zona pellucida because of its inadequate activation [123]. Although many different protocols and medium substitutions were tested, there was high variability among the results. Furthermore, obtaining a large number of oocytes for experimental purposes is quite challenging. Due to the high economic value of individual animals, it is more than necessary to improve the equine IVF methodology to be more functional and transferable between individual laboratories. Currently used methods are oocyte/embryo transfer and Intracytoplasmic Sperm Injection (ICSI) of oocytes recovered from slaughtered or live mares much more than classic IVF [124]. Although there are many differences, equine and human development have also a lot in common. The most important similarities are follicular dynamics, mono-ovulation, and kinetics of embryo development. Both horses and humans are reproductively active in the older age and suffer from rising overweight and obesity concerns. Taken together, besides pigs and cattle, horses could also serve as suitable model organisms for human-assisted reproduction [125].

Some of the ordinarily used supplementation with positive effects on IVM of cattle or pigs, have not been reported to have the same impact on equine. For example, although cysteamine supplementation to the maturation medium does not result in an improved maturation rate or IVF [126], it has been observed to enhance the bovine blastocyst rate [65]. On the contrary, supplementation of EGF to equine maturation media improved IVM rates as well as in bovine and porcine IVM [45]. Also, supplementation with β-mercaptoethanol during equine IVM did not affect maturation rates and cleavage rates after ICSI [127]. Although maturation rates of bovine were also not

increased, the quality and developmental competence of embryos originating from these oocytes were improved [128].

Higher equine maturation rates were observed also when the Tissue Culture Medium (TCM) was supplemented with FBS. TCM can help regulate hormonal imbalances and improve overall reproductive health, while FBS provides essential nutrients and growth factors necessary for cell growth and development. The combination of TCM with FBS improved oocyte maturation rates compared to traditional culture media (75.7 vs. 52.2%). FBS presence in maturation media also induced equine sperm capacitation, however, IVF were unsuccessful [43].

7. Conclusion

In conclusion of this overview, the use of maturation and culture media enriched with growth factors, antioxidants, and hormones has shown promising results in improving the success rate of in vitro embryo production. The inclusion of these components in the maturation medium can improve the quality of oocytes, support oocyte maturation, and influence the success of embryogenesis. However, it is important to note that the optimal combination and concentration of these components may vary depending on individual circumstances such as species, breed, age, and reproductive history. Further research is needed to fully understand the mechanisms of their effects and to develop more effective protocols for in vitro embryo production. Nonetheless, the use of these components has great potential for improving reproductive efficiency and genetic progress in in vitro embryo production programs.

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References

[1] Viana JH. 2019 statistics of embryo production and transfer in domestic farm animals. Embryo Technology Newsletter. 2020;**38**(4):1-15

[2] Viana JH. 2020 statistics of embryo production and transfer in domestic farm animals. Embryo Technology Newsletter. 2021;**39**(4):1-14

[3] Viana JH. 2021 statistics of embryo production and transfer in domestic farm animals. Embryo Technology Newsletter. 2022;**40**(4):1-17

[4] Hatırnaz Ş, Ata B, Saynur Hatırnaz E, Dahan MH, Tannus S, Tan J, et al. Oocyte in vitro maturation: A sytematic review. Journal of Turkish Society of Obstetric and Gynecology. 2018;**15**:112-125. DOI: 10.4274/tjod.23911

[5] Bahrami M, Cottee PA. Culture conditions for in vitro maturation of oocytes – A review. Reproduction and Breeding. 2022;**2**:31-36. DOI: 10.1016/j.repbre.2022.04.001

[6] Silva AFB, Lima LF, Morais ANP, Lienou LL, Watanabe YF, Joaquim DC, et al. Oocyte in vitro maturation with eugenol improves the medium antioxidant capacity and total cell number per blastocyst. Theriogenology. 2022;**192**:109-115. DOI: 10.1016/j.theriogenology.2022.08.024

[7] Dang-Nguyen TQ, Somfai T, Haraguchi S, Kikuchi K, Tajima A, Kanai Y, et al. In vitro production of porcine embryos: Current status, future perspectives and alternative applications: In vitro production of porcine embryos. Animal Science Journal. 2011;**82**:374-382. DOI: 10.1111/j.1740-0929.2011.00883.x

[8] de Souza-Fabjan JMG, Panneau B, Duffard N, Locatelli Y, de Figueiredo JR, de Figueirêdo Freitas VJ, et al. In vitro production of small ruminant embryos: Late improvements and further research. Theriogenology. 2014;**81**:1149-1162. DOI: 10.1016/j.theriogenology.2014.02.001

[9] Morohaku K. A way for *in vitro/ex vivo* egg production in mammals. The Journal of Reproduction and Development. 2019;**65**:281-287. DOI: 10.1262/jrd.2019-024

[10] Xu J, Zelinski MB. Oocyte quality following in vitro follicle development. Biology of Reproduction. 2022;**106**:291-315. DOI: 10.1093/biolre/ioab242

[11] de Figueiredo JR, Cadenas J, de Lima LF, Santos RR. Advances in in vitro folliculogenesis in domestic ruminants. Animal Reproduction. 2019;**16**:52-65. DOI: 10.21451/1984-3143-AR2018-0123

[12] Conti M, Franciosi F. Acquisition of oocyte competence to develop as an embryo: Integrated nuclear and cytoplasmic events. Human Reproduction Update. 2018;**24**:245-266. DOI: 10.1093/humupd/dmx040

[13] Sanders JR, Jones KT. Regulation of the meiotic divisions of mammalian oocytes and eggs. Biochemical Society Transactions. 2018;**46**:797-806. DOI: 10.1042/BST20170493

[14] Pan B, Li J. The art of oocyte meiotic arrest regulation. Reproductive Biology and Endocrinology. 2019;**17**:8. DOI: 10.1186/s12958-018-0445-8

[15] Macaulay AD, Gilbert I, Scantland S, Fournier E, Ashkar F, Bastien A, et al. Cumulus cell transcripts transit to the bovine oocyte in preparation for Maturation. Biology of Reproduction.

2016;**94**(1):1-25. DOI: 10.1095/biolreprod.114.127571

[16] Kordowitzki P, Sokołowska G, Wasielak-Politowska M, Skowronska A, Skowronski MT. Pannexins and Connexins: Their relevance for oocyte developmental competence. International Journal of Molecular Sciences. 2021;**22**:5918. DOI: 10.3390/ijms22115918

[17] Filatov M, Khramova Y, Semenova M. Molecular mechanisms of prophase I meiotic arrest maintenance and meiotic resumption in mammalian oocytes. Reproductive Sciences (Thousand Oaks, Calif.). 2019;**26**:1519-1537. DOI: 10.1177/1933719118765974

[18] Lavrov DV, Pett W. Animal mitochondrial DNA as we Do not know it: mt-genome organization and evolution in Nonbilaterian lineages. Genome Biology and Evolution. 2016;**8**:2896-2913. DOI: 10.1093/gbe/evw195

[19] Hardy MLM, Day ML, Morris MB. Redox regulation and oxidative stress in mammalian oocytes and embryos developed In vivo and In vitro. International Journal of Environmental Research and Public Health. 2021;**18**:11374. DOI: 10.3390/ijerph182111374

[20] Arias ME, Vargas T, Gallardo V, Aguila L, Felmer R. Simple and efficient chemically defined In vitro maturation and embryo culture system for bovine embryos. Animals. 2022;**12**:3057. DOI: 10.3390/ani12213057

[21] Pereira GR, Lorenzo PL, Carneiro GF, Ball BA, Pegoraro LMC, Pimentel CA, et al. Influence of equine growth hormone, insulin-like growth factor-I and its interaction with gonadotropins on in vitro maturation and cytoskeleton morphology in equine oocytes. Animal. 2013;**7**:1493-1499. DOI: 10.1017/S175173111300116X

[22] Lonergan P, Fair T. Maturation of oocytes in vitro. Annual Review of Animal Biosciences. 2016;**4**:255-268. DOI: 10.1146/annurev-animal-022114-110822

[23] Murin M, Nemcova L, Bartkova A, Gad A, Lucas-Hahn A, Strejcek F, et al. Porcine oocytes matured in a chemically defined medium are transcriptionally active. Theriogenology. 2023;**203**:89-98. DOI: 10.1016/j.theriogenology.2023.03.019

[24] Saeed-Zidane M, Tesfaye D, Mohammed Shaker Y, Tholen E, Neuhoff C, Rings F, et al. Hyaluronic acid and epidermal growth factor improved the bovine embryo quality by regulating the DNA methylation and expression patterns of the focal adhesion pathway. PLoS One. 2019;**14**:e0223753. DOI: 10.1371/journal.pone.0223753

[25] Kinterova V, Kanka J, Petruskova V, Toralova T. Inhibition of Skp1-Cullin-F-box complexes during bovine oocyte maturation and preimplantation development leads to delayed development of embryos†. Biology of Reproduction. 2019;**100**:896-906. DOI: 10.1093/biolre/ioy254

[26] Guimarães ALS, Pereira SA, Leme LO, Dode MAN. Evaluation of the simulated physiological oocyte maturation system for improving bovine in vitro embryo production. Theriogenology. 2015;**83**:52-57. DOI: 10.1016/j.theriogenology.2014.07.042

[27] Crocomo LF, Filho WCM, Sudano MJ, Paschoal DM, da Alvarenga FCL, Bicudo SD. Effect of roscovitine and cycloheximide on

ultrastructure of sheep oocytes. Small Ruminant Research. 2013;**109**:156-162. DOI: 10.1016/j.smallrumres.2012.07.006

[28] Appeltant R, Somfai T, Maes D, Van Soom A, Kikuchi K. Porcine oocyte maturation *in vitro*: Role of cAMP and oocyte-secreted factors – A practical approach. The Journal of Reproduction and Development. 2016;**62**:439-449. DOI: 10.1262/jrd.2016-016

[29] Procházka R, Němcová L, Nagyová E, Scsuková S, Mlynarčíková A. Development of functional LH receptors on pig cumulus-oocyte complexes cultured in vitro by a novel two-step culture system. Molecular Reproduction and Development. 2009;**76**:751-761. DOI: 10.1002/mrd.21039

[30] Gupta A, Tiwari M, Prasad S, Chaube SK. Role of cyclic nucleotide Phosphodiesterases during meiotic resumption from Diplotene arrest in mammalian oocytes: P hosphodiesterases and oocyte meiotic resumption. Journal of Cellular Biochemistry. 2017;**118**:446-452. DOI: 10.1002/jcb.25748

[31] Menino AR, Wright RW. Development of one-cell porcine embryos in two culture Systems3. Journal of Animal Science. 1982;**54**:583-588. DOI: 10.2527/jas1982.543583x

[32] Kim H, Lee G, Hyun S, Lee S, Nam D, Jeong Y, et al. Improved in vitro development of porcine embryos with different energy substrates and serum. Theriogenology. 2004;**61**:1381-1393. DOI: 10.1016/j.theriogenology.2003.08.012

[33] Prather RS, Redel BK, Whitworth KM, Zhao M-T. Genomic profiling to improve embryogenesis in the pig. Animal Reproduction Science. 2014;**149**:39-45. DOI: 10.1016/j.anireprosci.2014.04.017

[34] Usman SF, Shuaibu IR, Durojaiye K, Medugu N, Iregbu KC. The presence of microorganisms in follicular fluid and its effect on the outcome of in vitro fertilization-embryo transfer (IVF-ET) treatment cycles. PLoS One. 2021;**16**:e0246644. DOI: 10.1371/journal.pone.0246644

[35] Summers MC, Biggers JD. Chemically defined media and the culture of mammalian preimplantation embryos: Historical perspective and current issues. Human Reproduction Update. 2003;**9**:557-582. DOI: 10.1093/humupd/dmg039

[36] Bradley J, Swann K. Mitochondria and lipid metabolism in mammalian oocytes and early embryos. The International Journal of Developmental Biology. 2019;**63**:93-103. DOI: 10.1387/ijdb.180355ks

[37] Chen X-Y, Li Q-W, Zhang S-S, Han Z-S, Zhao R, Wu S-Y, et al. Effects of ovarian cortex cell co-culture during in vitro maturation on porcine oocytes maturation, fertilization and embryo development. Animal Reproduction Science. 2007;**99**:306-316. DOI: 10.1016/j.anireprosci.2006.05.007

[38] Virant-Klun I, Bauer Ch, Stahlberg A, Kubista M. Human oocyte maturation in vitro is improved by co-culture with cumulus cells from mature oocytes. Reproductive BioMedicine Online. 2018;**36**(5):508-523. DOI: 10.1016/j.rbmo.2018.01.011

[39] Khurana NK, Niemann H. Energy metabolism in preimplantation bovine embryos derived In vitro or In vivo. Biology of Reproduction. 2000;**62**:847-856. DOI: 10.1095/biolreprod62.4.847

[40] Silva BR, Silva JRV. Mechanisms of action of non-enzymatic antioxidants to control oxidative stress during in

vitro follicle growth, oocyte maturation, and embryo development. Animal Reproduction Science. 2023;**249**:107186. DOI: 10.1016/j.anireprosci.2022.107186

[41] Sovernigo T, Adona P, Monzani P, Guemra S, Barros F, Lopes F, et al. Effects of supplementation of medium with different antioxidants during in vitro maturation of bovine oocytes on subsequent embryo production. Reproduction in Domestic Animals. 2017;**52**:561-569. DOI: 10.1111/rda.12946

[42] Currin L, Glanzner WG, Gutierrez K, de Macedo MP, Guay V, Baldassarre H, et al. Optimizing swine in vitro embryo production with growth factor and antioxidant supplementation during oocyte maturation. Theriogenology. 2022;**194**:133-143. DOI: 10.1016/j.theriogenology.2022.10.005

[43] González-Fernández L, Macedo S, Lopes J, Rocha A, Macías-García B. Effect of different media and protein source on equine gametes: Potential impact during *In vitro* fertilization. Reproduction in Domestic Animals. 2015;**50**:1039-1046. DOI: 10.1111/rda.12634

[44] Soto-Moreno EJ, Balboula A, Spinka C, Rivera RM. Serum supplementation during bovine embryo culture affects their development and proliferation through macroautophagy and endoplasmic reticulum stress regulation. PLoS One. 2021;**16**:e0260123. DOI: 10.1371/journal.pone.0260123

[45] Lorenzo PL, Liu IKM, Carneiro GF, Conley AJ, Enders AC. Equine oocyte maturation with epidermal growth factor. Equine Veterinary Journal. 2010;**34**:378-382. DOI: 10.2746/042516402776249065

[46] Neira JA, Tainturier D, Peña MA, Martal J. Effect of the association of IGF-I, IGF-II, bFGF, TGF-β1, GM-CSF, and LIF on the development of bovine embryos produced in vitro. Theriogenology. 2010;**73**:595-604. DOI: 10.1016/j.theriogenology.2009.10.015

[47] Ptak G, Lopes F, Matsukawa K, Tischner M, Loi P. Leukaemia inhibitory factor enhances sheep fertilization in vitro via an influence on the oocyte. Theriogenology. 2006;**65**:1891-1899. DOI: 10.1016/j.theriogenology. 2005.10.018

[48] An L, Liu J, Du Y, Liu Z, Zhang F, Liu Y, et al. Synergistic effect of cysteamine, leukemia inhibitory factor, and Y27632 on goat oocyte maturation and embryo development in vitro. Theriogenology. 2018;**108**:56-62. DOI: 10.1016/j.theriogenology.2017.11.028

[49] Fields SD, Hansen PJ, Ealy AD. Fibroblast growth factor requirements for in vitro development of bovine embryos. Theriogenology. 2011;**75**:1466-1475. DOI: 10.1016/j.theriogenology.2010.12.007

[50] Mondal S, Mor A, Reddy IJ, Nandi S, Parameswaragupta PS. Effect of fibroblast growth factor 2 (FGF2) and insulin transferrin selenium (ITS) on In vitro maturation, fertilization and embryo development in sheep. Brazilian Archives of Biology and Technology. 2015;**58**:521-525. DOI: 10.1590/S1516-8913201500059

[51] Subbiahanadar Chelladurai K, Selvan Christyraj JD, Rajagopalan K, Yesudhason BV, Venkatachalam S, Mohan M, et al. Alternative to FBS in animal cell culture - An overview and future perspective. Heliyon. 2021;**7**:e07686. DOI: 10.1016/j.heliyon.2021.e07686

[52] Kang J-T, Koo O-J, Kwon D-K, Park H-J, Jang G, Kang S-K, et al. Effects of melatonin on in vitro maturation of porcine oocyte and expression of melatonin receptor RNA in cumulus and granulosa cells. Journal

of Pineal Research. 2009;**46**:22-28. DOI: 10.1111/j.1600-079X.2008.00602.x

[53] Do L, Shibata Y, Taniguchi M, Nii M, Nguyen T, Tanihara F, et al. Melatonin supplementation during *In vitro* maturation and development supports the development of porcine embryos. Reproduction in Domestic Animals. 2015;**50**:1054-1058. DOI: 10.1111/rda.12607

[54] Shi J-M, Tian X-Z, Zhou G-B, Wang L, Gao C, Zhu S-E, et al. Melatonin exists in porcine follicular fluid and improves in vitro maturation and parthenogenetic development of porcine oocytes. Journal of Pineal Research. 2009;**47**:318-323. DOI: 10.1111/j.1600-079X.2009.00717.x

[55] Nakano M, Kato Y, Tsunoda Y. Effect of melatonin treatment on the developmental potential of parthenogenetic and somatic cell nuclear-transferred porcine oocytes *in vitro*. Zygote. 2012;**20**:199-207. DOI: 10.1017/S0967199411000190

[56] El-Raey M, Geshi M, Somfai T, Kaneda M, Hirako M, Abdel-Ghaffar AE, et al. Evidence of melatonin synthesis in the cumulus oocyte complexes and its role in enhancing oocyte maturation in vitro in cattle: M elatonin effects on cattle oocytes. Molecular Reproduction and Development. 2011;**78**:250-262. DOI: 10.1002/mrd.21295

[57] Pang Y, Zhao S, Sun Y, Jiang X, Hao H, Du W, et al. Protective effects of melatonin on the *in vitro* developmental competence of bovine oocytes. Animal Science Journal. 2018;**89**:648-660. DOI: 10.1111/asj.12970

[58] Wang F, Tian X, Zhou Y, Tan D, Zhu S, Dai Y, et al. Melatonin improves the quality of In vitro produced (IVP) bovine embryos: Implications for blastocyst development, Cryotolerance, and modifications of relevant gene expression. PLoS One. 2014;**9**:e93641. DOI: 10.1371/journal.pone.0093641

[59] Yang M, Tao J, Chai M, Wu H, Wang J, Li G, et al. Melatonin improves the quality of inferior bovine oocytes and promoted their subsequent IVF embryo development: Mechanisms and results. Molecules. 2017;**22**:2059. DOI: 10.3390/molecules22122059

[60] Casao A, Abecia JA, Pérez JAC, Blanco TM, Vázquez MI, Forcada F. The effects of melatonin on in vitro oocyte competence and embryo development in sheep. Spanish Journal of Agricultural Research. 2010;**8**:35-41. DOI: 10.5424/sjar/2010081-1141

[61] Abecia J-A, Forcada F, Vázquez M-I, Muiño-Blanco T, Cebrián-Pérez JA, Pérez-Pe R, et al. Role of melatonin on embryo viability in sheep. Reproduction, Fertility, and Development. 2019;**31**:82-92. DOI: 10.1071/RD18308

[62] Chen Y, Shan X, Jiang H, Guo Z. Exogenous melatonin directly and indirectly influences sheep oocytes. Frontiers in Veterinary Science. 2022;**9**:903195. DOI: 10.3389/fvets.2022.903195

[63] Soto-Heras S, Roura M, Catalá MG, Menéndez-Blanco I, Izquierdo D, Fouladi-Nashta AA, et al. Beneficial effects of melatonin on in vitro embryo production from juvenile goat oocytes. Reproduction, Fertility, and Development. 2018;**30**:253. DOI: 10.1071/RD17170

[64] Marei WF, Ghafari F, Fouladi-Nashta AA. Role of hyaluronic acid in maturation and further early embryo development of bovine oocytes. Theriogenology. 2012;**78**:670-677. DOI: 10.1016/j.theriogenology.2012.03.013

[65] de Matos DG, Furnus CC, Moses DF, Baldassarre H. Effect of cysteamine on glutathione level and developmental capacity of bovine oocyte matured in vitro. Molecular Reproduction and Development. 1995;**42**:432-436. DOI: 10.1002/mrd.1080420409

[66] Tareq KMA, Akter QS, Khandoker MAMY, Tsujii H. Selenium and vitamin E improve the in vitro maturation, fertilization and culture to blastocyst of porcine oocytes. The Journal of Reproduction and Development. 2012;**58**:621-628. DOI: 10.1262/jrd.2012-064

[67] Almubarak AM, Kim E, Yu I-J, Jeon Y. Supplementation with niacin during in vitro maturation improves the quality of porcine embryos. Theriogenology. 2021;**169**:36-46. DOI: 10.1016/j.theriogenology.2021.04.005

[68] Lowe JL, Bartolac LK, Bathgate R, Grupen CG. Supplementation of culture medium with L-carnitine improves the development and cryotolerance of in vitro-produced porcine embryos. Reproduction, Fertility, and Development. 2017;**29**:2357. DOI: 10.1071/RD16442

[69] Jiang W-J, Yao X-R, Zhao Y-H, Gao Q-S, Jin Q-G, Li Y-H, et al. L-carnitine prevents bovine oocyte aging and promotes subsequent embryonic development. The Journal of Reproduction and Development. 2019;**65**:499-506. DOI: 10.1262/jrd.2019-046

[70] Knitlova D, Hulinska P, Jeseta M, Hanzalova K, Kempisty B, Machatkova M. Supplementation of l-carnitine during in vitro maturation improves embryo development from less competent bovine oocytes. Theriogenology. 2017;**102**:16-22. DOI: 10.1016/j.theriogenology.2017.06.025

[71] Mishra A, Reddy IJ, Gupta PSP, Mondal S. L-carnitine mediated reduction in oxidative stress and alteration in transcript level of antioxidant enzymes in sheep embryos produced In vitro. Reproduction in Domestic Animals = Zuchthygiene. 2016;**51**:311-321. DOI: 10.1111/rda.12682

[72] Wang X, Zhu X, Liang X, Xu H, Liao Y, Lu K, et al. Effects of resveratrol on in vitro maturation of porcine oocytes and subsequent early embryonic development following somatic cell nuclear transfer. Reproduction in Domestic Animals. 2019;**54**:1195-1205. DOI: 10.1111/rda.13493

[73] Kwak S-S, Cheong S-A, Jeon Y, Lee E, Choi K-C, Jeung E-B, et al. The effects of resveratrol on porcine oocyte in vitro maturation and subsequent embryonic development after parthenogenetic activation and in vitro fertilization. Theriogenology. 2012;**78**:86-101. DOI: 10.1016/j.theriogenology.2012.01.024

[74] Zabihi A, Shabankareh HK, Hajarian H, Foroutanifar S. Resveratrol addition to in vitro maturation and in vitro culture media enhances developmental competence of sheep embryos. Domestic Animal Endocrinology. 2019;**68**:25-31. DOI: 10.1016/j.domaniend.2018.12.010

[75] Piras A-R, Menéndez-Blanco I, Soto-Heras S, Catalá M-G, Izquierdo D, Bogliolo L, et al. Resveratrol supplementation during in vitro maturation improves embryo development of prepubertal goat oocytes selected by brilliant cresyl blue staining. The Journal of Reproduction and Development. 2019;**65**:113-120. DOI: 10.1262/jrd.2018-077

[76] Xiang D-C, Jia B-Y, Fu X-W, Guo J-X, Hong Q-H, Quan G-B, et al. Role of

astaxanthin as an efficient antioxidant on the in vitro maturation and vitrification of porcine oocytes. Theriogenology. 2021;**167**:13-23. DOI: 10.1016/j.theriogenology.2021.03.006

[77] Do LTK, Luu VV, Morita Y, Taniguchi M, Nii M, Peter AT, et al. Astaxanthin present in the maturation medium reduces negative effects of heat shock on the developmental competence of porcine oocytes. Reproductive Biology. 2015;**15**:86-93. DOI: 10.1016/j.repbio.2015.01.002

[78] dos Santos M-SA, Guimarães ALS, Fidelis AAG, Franco MM, Dode MAN. The use of insulin-transferrin-selenium (ITS), and folic acid on individual in vitro embryo culture systems in cattle. Theriogenology. 2022;**184**:153-161. DOI: 10.1016/j.theriogenology.2022.03.008

[79] Residiwati G, Azari-Dolatabad N, Tuska HSA, Sidi S, Van Damme P, Benedetti C, et al. Effect of lycopene supplementation to bovine oocytes exposed to heat shock during in vitro maturation. Theriogenology. 2021;**173**:48-55. DOI: 10.1016/j.theriogenology.2021.07.014

[80] Chowdhury MMR, Choi B-H, Khan I, Lee K-L, Mesalam A, Song S-H, et al. Supplementation of lycopene in maturation media improves bovine embryo quality in vitro. Theriogenology. 2017;**103**:173-184. DOI: 10.1016/j.theriogenology.2017.08.003

[81] Sidi S, Pascottini OB, Angel-Velez D, Azari-Dolatabad N, Pavani KC, Residiwati G, et al. Lycopene supplementation to serum-free maturation medium improves In vitro bovine embryo development and quality and modulates embryonic transcriptomic profile. Antioxidants. 2022;**11**:344. DOI: 10.3390/antiox11020344

[82] Ynsaurralde-Rivolta AE, Suvá M, Luchetti CG, Bevacqua RJ, Munilla S, Rodriguez-Alvarez L, et al. DMSO supplementation during in vitro maturation of bovine oocytes improves blastocyst rate and quality. Theriogenology. 2020;**148**:140-148. DOI: 10.1016/j.theriogenology.2020.02.045

[83] Tian H, Qi Q, Yan F, Wang C, Hou F, Ren W, et al. Enhancing the developmental competence of prepubertal lamb oocytes by supplementing the in vitro maturation medium with sericin and the fibroblast growth factor 2 - leukemia inhibitory factor - insulin-like growth factor 1 combination. Theriogenology. 2021;**159**:13-19. DOI: 10.1016/j.theriogenology.2020.10.019

[84] Aghaz F, Hajarian H, Shabankareh HK, Abdolmohammadi A. Effect of sericin supplementation in maturation medium on cumulus cell expansion, oocyte nuclear maturation, and subsequent embryo development in Sanjabi ewes during the breeding season. Theriogenology. 2015;**84**:1631-1635. DOI: 10.1016/j.theriogenology.2015.08.013

[85] Hansen JM, Harris C. Glutathione during embryonic development. Biochim Biophys Acta BBA - Gen Subj. 2015;**1850**:1527-1542. DOI: 10.1016/j.bbagen.2014.12.001

[86] Sun W-J, Pang Y-W, Liu Y, Hao H-S, Zhao X-M, Qin T, et al. Exogenous glutathione supplementation in culture medium improves the bovine embryo development after in vitro fertilization. Theriogenology. 2015;**84**:716-723. DOI: 10.1016/j.theriogenology.2015.05.001

[87] Ren J, Hao Y, Liu Z, Li S, Wang C, Wang B, et al. Effect of exogenous glutathione supplementation on the in vitro developmental competence of

ovine oocytes. Theriogenology. 2021;**173**:144-155. DOI: 10.1016/j.theriogenology.2021.07.025

[88] Veshkini A, Mohammadi-Sangcheshmeh A, Ghanem N, Abazari-Kia AH, Mottaghi E, Kamaledini R, et al. Oocyte maturation with royal jelly increases embryo development and reduces apoptosis in goats. Animal Reproduction. 2018;**15**:124-134. DOI: 10.21451/1984-3143-2017-AR986

[89] Veshkini A, Khadem AA, Mohammadi-Sangcheshmeh A, Alamouti AA, Soleimani M, Gastal EL. Linolenic acid improves oocyte developmental competence and decreases apoptosis of in vitro-produced blastocysts in goat. Zygote (Cambridge, England). 2016;**24**:537-548. DOI: 10.1017/S0967199415000507

[90] Mishra A, Reddy IJ, Dhali A, Javvaji PK. L-Ergothioneine improves the developmental potential of in vitro sheep embryos without influencing OCTN1-mediated cross-membrane transcript expression. Zygote (Cambridge, England). 2018;**26**:149-161. DOI: 10.1017/S0967199418000047

[91] Guimarães ALS, Pereira SA, Diógenes MN, Dode MAN. Effect of insulin–transferrin–selenium (ITS) and L -ascorbic acid (AA) during *in vitro* maturation on *in vitro* bovine embryo development. Zygote. 2016;**24**:890-899. DOI: 10.1017/S0967199416000228

[92] Gutierrez K, Dicks N, Glanzner WG, Agellon LB, Bordignon V. Efficacy of the porcine species in biomedical research. Frontiers in Genetics. 2015;**6**(293):1-9. DOI: 10.3389/fgene.2015.00293

[93] Walters EM, Prather RS. Advancing swine models for human health and diseases. Missouri Medicine. 2013;**110**:212-215

[94] Cheng WTK, Moor RM, Polge C. In vitro fertilization of pig and sheep oocytes matured in vivo and in vitro. Theriogenology. 1986;**25**:146. DOI: 10.1016/0093-691X(86)90200-1

[95] Mahanta N, Bhuyan D, Kumar S, Biswas RK, Dutta DJ, Deka BC, et al. Effect of growth factor on in-vitro maturation of porcine oocyte. Indian Journal of Animal Research. 2018;**52**(3):353-357. DOI: 10.18805/ijar.B-3202

[96] Wu J, Xu B, Wang W. Effects of luteinizing hormone and follicle stimulating hormone on the developmental competence of porcine preantral follicle oocytes grown in vitro. Journal of Assisted Reproduction and Genetics. 2007;**24**:419-424. DOI: 10.1007/s10815-007-9154-5

[97] Silvestre MA, Alfonso J, Garcia-Mengual E, Salvador I, Duque CC, Molina I. Effect of recombinant human follicle-stimulating hormone and luteinizing hormone on in vitro maturation of porcine oocytes evaluated by the subsequent in vitro development of embryos obtained by in vitro fertilization, intracytoplasmic sperm injection, or parthenogenetic activation. Journal of Animal Science. 2007;**85**:1156-1160. DOI: 10.2527/jas2006-645

[98] León J, Escames G, Rodríguez MI, López LC, Tapias V, Entrena A, et al. Inhibition of neuronal nitric oxide synthase activity by N1-acetyl-5-methoxykynuramine, a brain metabolite of melatonin: Melatonin metabolites and brain nNOS. Journal of Neurochemistry. 2006;**98**:2023-2033. DOI: 10.1111/j.1471-4159.2006.04029.x

[99] Noda Y, Mori A, Liburdy R, Packer L. Melatonin and its precursors scavenge nitric oxide. Journal of Pineal Research. 1999;**27**:159-163. DOI: 10.1111/j.1600-079X.1999.tb00611.x

[100] Khan R, Jiang X, Hameed U, Shi Q. Role of lipid metabolism and Signaling in mammalian oocyte maturation, quality, and Acquisition of Competence. Frontiers in Cell and Development Biology. 2021;**9**:639704. DOI: 10.3389/fcell.2021.639704

[101] Hoyos-Marulanda V, Alves BS, Rosa PRA, Vieira AD, Gasperin BG, Mondadori RG, et al. Effects of polyunsaturated fatty acids on the development of pig oocytes in vitro following parthenogenetic activation and on the lipid content of oocytes and embryos. Animal Reproduction Science. 2019;**205**:150-155. DOI: 10.1016/j.anireprosci.2019.05.003

[102] Wu G-Q, Jia B-Y, Li J-J, Fu X-W, Zhou G-B, Hou Y-P, et al. L-carnitine enhances oocyte maturation and development of parthenogenetic embryos in pigs. Theriogenology. 2011;**76**:785-793. DOI: 10.1016/j.theriogenology.2011.04.011

[103] Li J, Liu L, Weng J, Yin T, Yang J, Feng HL. Biological roles of L-carnitine in oocyte and early embryo development. Molecular Reproduction and Development. 2021;**88**:673-685. DOI: 10.1002/mrd.23542

[104] Granleese T, Clark SA, Swan AA, van der Werf JHJ. Increased genetic gains in sheep, beef and dairy breeding programs from using female reproductive technologies combined with optimal contribution selection and genomic breeding values. Genetics, Selection, Evolution. 2015;**47**:70. DOI: 10.1186/s12711-015-0151-3

[105] Goto K, Kajihara Y, Kosaka S, Koba M, Nakanishi Y, Ogawa K. Pregnancies after co-culture of cumulus cells with bovine embryos derived from in-vitro fertilization of in-vitro matured follicular oocytes. Reproduction. 1988;**83**:753-758. DOI: 10.1530/jrf.0.0830753

[106] Ealy AD, Wooldridge LK, McCoski SR. Board invited review: Post-transfer consequences of in vitro-produced embryos in cattle. Journal of Animal Science. 2019;**97**:2555-2568. DOI: 10.1093/jas/skz116

[107] Wooldridge LK, Keane JA, Rhoads ML, Ealy AD. Bioactive supplements influencing bovine in vitro embryo development. Journal of Animal Science. 2022;**100**:skac091. DOI: 10.1093/jas/skac091

[108] Murillo A, Muñoz M, Martín-González D, Carrocera S, Martínez-Nistal A, Gómez E. Low serum concentration in bovine embryo culture enhances early blastocyst rates on Day-6 with quality traits in the expanded blastocyst stage similar to BSA-cultured embryos. Reproductive Biology. 2017;**17**:162-171. DOI: 10.1016/j.repbio.2017.04.002

[109] Lim JM, Liou SS, Hansel W. Intracytoplasmic glutathione concentration and the role of β-mercaptoethanol in preimplantation development of bovine embryos. Theriogenology. 1996;**46**:429-439. DOI: 10.1016/0093-691X(96)00165-3

[110] Kim M-J, Gim G-M, Jang G. Supplement of secreted recombinant low molecular weight human fibroblast growth factor 2 in culture media enhances in vitro bovine maturation. Research in Veterinary Science. 2022;**153**:27-34. DOI: 10.1016/j.rvsc.2022.10.007

[111] Yang S, Yang Y, Hao H, Du W, Pang Y, Zhao S, et al. Supplementation of EGF, IGF-1, and Connexin 37 in IVM medium significantly improved the maturation of bovine oocytes and Vitrification of their IVF blastocysts.

Genes. 2022;**13**:805. DOI: 10.3390/genes13050805

[112] Anchordoquy JP, Lizarraga RM, Anchordoquy JM, Nikoloff N, Rosa DE, Fabra MC, et al. Effect of cysteine, glutamate and glycine supplementation to in vitro fertilization medium during bovine early embryo development. Reproductive Biology. 2019;**19**:349-355. DOI: 10.1016/j.repbio.2019.10.002

[113] Gasparrini B, Boccia L, Marchandise J, Di Palo R, George F, Donnay I, et al. Enrichment of in vitro maturation medium for buffalo (Bubalus bubalis) oocytes with thiol compounds: Effects of cystine on glutathione synthesis and embryo development. Theriogenology. 2006;**65**:275-287. DOI: 10.1016/j.theriogenology.2005.05.036

[114] de Matos DG, Gasparrini B, Pasqualini SR, Thompson JG. Effect of glutathione synthesis stimulation during in vitro maturation of ovine oocytes on embryo development and intracellular peroxide content. Theriogenology. 2002;**57**:1443-1451. DOI: 10.1016/S0093-691X(02)00643-X

[115] Whitaker BD, Knight JW. Exogenous γ-glutamyl cycle compounds supplemented to in vitro maturation medium influence in vitro fertilization, culture, and viability parameters of porcine oocytes and embryos. Theriogenology. 2004;**62**:311-322. DOI: 10.1016/j.theriogenology.2003.10.014

[116] Zullo G, Albero G, Neglia G, De Canditiis C, Bifulco G, Campanile G, et al. L-ergothioneine supplementation during culture improves quality of bovine in vitro–produced embryos. Theriogenology. 2016;**85**:688-697. DOI: 10.1016/j.theriogenology.2015.10.008

[117] Kalds P, Zhou S, Cai B, Liu J, Wang Y, Petersen B, et al. Sheep and goat genome engineering: From random Transgenesis to the CRISPR era. Frontiers in Genetics. 2019;**10**:750. DOI: 10.3389/fgene.2019.00750

[118] Yuan Y, Spate LD, Redel BK, Tian Y, Zhou J, Prather RS, et al. Quadrupling efficiency in production of genetically modified pigs through improved oocyte maturation. Proceedings of the National Academy of Sciences of the United States of America. 2017;**114**:E5796-E5804. DOI: 10.1073/pnas.1703998114

[119] Wang DH, Ren J, Zhou CJ, Han Z, Wang L, Liang CG. Supplementation with CTGF, SDF1, NGF, and HGF promotes ovine in vitro oocyte maturation and early embryo development. Domestic Animal Endocrinology. 2018;**65**:38-48. DOI: 10.1016/j.domaniend.2018.05.003

[120] Fathi M, Elkarmoty AF. Effect of adding growth factors during in vitro maturation on the developmental potentials of ewe oocytes selected by brilliant cresyl blue staining. Veterinary World. 2021;**14**:452-456. DOI: 10.14202/vetworld.2021.452-456

[121] Sofi KA, Khan MZ, Islam R, Lone FA. Effect of cysteamine and epidermal growth factor supplementation on the in vitro maturation rate of ovine oocytes. Small Ruminant Research. 2011;**96**:191-194. DOI: 10.1016/j.smallrumres.2010.10.012

[122] Saeedabadi S, Abazari-Kia AH, Rajabi H, Parivar K, Salehi M. Melatonin improves the developmental competence of goat oocytes. International Journal of Fertility & Sterility. 2018;**12**(2):157-163. DOI: 10.22074/ijfs.2018.5204

[123] Leemans B, Gadella BM, Stout TAE, De Schauwer C, Nelis H, Hoogewijs M,

et al. Why doesn't conventional IVF work in the horse? The equine oviduct as a microenvironment for capacitation/fertilization. Reproduction. 2016;**152**:R233-R245. DOI: 10.1530/REP-16-0420

[124] Hinrichs K. Assisted reproduction techniques in the horse. Reproduction, Fertility, and Development. 2013;**25**:80. DOI: 10.1071/RD12263

[125] Benammar A, Derisoud E, Vialard F, Palmer E, Ayoubi JM, Poulain M, et al. The Mare: A pertinent model for human assisted reproductive technologies? Animals. 2021;**11**:2304. DOI: 10.3390/ani11082304

[126] Deleuze S, Dubois C, Caillaud M, Bruneau B, Goudet G, Duchamp G. Influence of Cysteamine on *In vitro* maturation, *In vitro* and *In vivo* fertilization of equine oocytes. Reproduction in Domestic Animals. 2010;**45**:1-7. DOI: 10.1111/j.1439-0531.2008.01122.x

[127] de Matos DG, Furnus CC. The importance of having high glutathione (GSH) level after bovine in vitro maturation on embryo development: Effect of β-mercaptoethanol, cysteine and cystine. Theriogenology. 2000;**53**:761-771. DOI: 10.1016/S0093-691X(99)00278-2

[128] Merlo B, Iacono E, Bucci D, Spinaci M, Galeati G, Mari G. Beta-mercaptoethanol supplementation of in vitro maturation medium does not influence nuclear and cytoplasmic maturation of equine oocytes. Reproduction in Domestic Animals. 2016;**51**:992-996. DOI: 10.1111/rda.12778