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The effects of culture media and media components on the development of rat embryos

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ABSTRACT

After in vitro culture of rat embryos, blastocyst rates are lower than the other species because of the embryonic block observed in the 2- or 4-cell stages in vitro. Optimal culture media and systems that provide variable physiologic needs in the different stages of rat embryos. The modifications of rat embryo culture media could have a positive effect on increasing the blastocyst rates. However, since the results of rat embryo studies are changed depending on factors like strains preferred, maintenance conditions and different commercial products added to the culture media, the success rate of producing healthy newborns for reproductive biotechnological studies has not yet reached the desired level by using current embryo culture media. Understanding the needs of rat embryos cultured from zygote to blastocyst stage in vitro is important for successful advanced studies such as cloning and transgenesis. The purpose of this review is the effects of different culture media and media components on the preimplantation stages of rat embryos and get a perspective for developing the culture media.

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Introduction

The laboratory rats are one of the most preferred species among laboratory animals for different research areas such as toxicology, biomedical engineering, biology, genetics, medicine and reproductive biotechnology (Iannaccone and Galat 2014, Hickman et al. 2017). There are similarities between rats and humans genetically, anatomically, and physiologically. Therefore, the rats can also contribute to cancer and other human disease studies (Iannaccone and Galat 2014, Agca 2019).

There are many difficulties due to the embryonic metabolism and culture conditions while developing the rat embryos from the zygote to the blastocyst stage. An embryonic developmental block occurs in in vitro culture and is generally caused during the zygotic gene activation phase (ZGA) which is happened in different embryonic stages for different mammalian embryos (Telford et al. 1990, Yamada and Nishikimi 1999) and ZGA happens in 2- or 4-cell stage in rat embryos (Mayer and Fritz 1974). To overcome in vitro embryonic developmental arrest, a lot of studies have been carried out (Zhang and Armstrong 1990, Kishi et al. 1991). On the other hand, the metabolism and developmental biology of rat embryos are not well understood because of the difficulties of getting high-quality fertile embryos through superovulation procedures (Miller and Armstrong 1981, Brison and Leese 1991). More efficient embryo culture media has been developed when starting to understand rat preimplantation embryo metabolism and physiology (Summers and Biggers 2003, Vajta et al. 2010, Men et al. 2023). To understand better the metabolism and in

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vitro culture needs of rat embryos, a retrospective examination should be done for rat embryo culture media in the present review, and it can help in getting perspective.

The overview of rat embryo culture media

The mammalian embryo culture media show similarity with physiologic salt solutions such as Earle Balanced Saline Solution, Tyrode solution and KRB (Krebs-Ringer bicarbonate); M16 media is based on Tyrode solution and Whitten's medium is derived from KRB (reviewed in Summers and Biggers 2003).

There was not any embryo culture medium that developed rat embryos successfully before the 90s (Miyoshi 2016), and some culture media has been improved with modifications after understanding the energy metabolism and physiology of mammalian embryos (Men et al. 2023). mKRB medium (modified Krebs-Ringer bicarbonate medium) has been developed by Toyoda and Chang (1974) and used as an in vitro fertilization medium in rats. Kishi et al. (1991) have used HECM-1 (chemically defined hamster 1-cell embryo culture media) to develop rat preimplantation embryos. Miyoshi et al. (1994) have developed mHECM-1 (modified hamster 1-cell embryo culture media) for rat embryo culture. Miyoshi et al. (1995a) has been studied on special media to develop rat embryos and designed R1ECM (rat 1-cell embryo culture medium). R1ECM has been improved by same group (Miyoshi et al. 1995b), and the media renamed as mR1ECM (modified rat 1-cell embryo culture medium). After the success of KSOM medium (Potassium simplex optimized media) in mouse embryo culture, KSOM-R was designed by Nakamura et al. (2016) for rat embryos. Also, M16 medium has been used experimentally to develop rat embryos (Popova et al. 2011).

Media components

Glucose and phosphate: Glucose and phosphate were thought to be the main reasons for the occurrence of developmental embryonic block of the preimplantation rat embryos cultured in vitro, although they are the components of oviduct fluid (Kishi et al. 1991, Miyoshi et al. 1994, Tsujii and Nakamura 1999). Moreover, glucose did not play a role in the rat embryos in the early division stages from 1-cell to morulae, neither promoting nor inhibiting effects have been shown (Miyoshi et al. 1994). However, preimplantation rat embryos in the later stages were affected positively by lower glucose concentrations in vitro. Some studies have shown that glucose uptake increased in the blastocyst stage for rat embryos (Brison and Leese 1991) and glucose enhanced the speed of embryonic development (Matsumoto and Sugawara 1995).

It has been reported that inorganic phosphate-included embryo culture media prevent the in vitro development of hamster and rat embryos critically (Schini and Bavister 1988, Yamada and Nishikimi 1999). Although low amounts of phosphate (0.001-0.01 µM) did not inhibit the embryonic development of rat embryos from zygote to blastocyst stage in the glucose-added HECM-1, 0.1 µM phosphate concentration blocked the development of 4-cell rat embryos to the morulae stage. The development of 2-cell rat embryos to the 4-cell stage was limited when increasing phosphate concentration, and 1-cell embryos did not proceed beyond to 2-cell stage in higher concentrations of phosphate (Miyoshi et al. 1994, Miyoshi et al. 1995a). It has also been reported that 1-cell rat embryos were not affected negatively by the low amount of phosphate and embryos, after they reached the 2-cell stage, developed to the blastocyst stage in the medium without phosphate (Matsumoto and Sugawara 1995). Miyoshi and Niwa (1997) however indicated that rat embryos beyond the 8-cell stage were not affected negatively by phosphate, and blastocyst formation and cell numbers increased in the culture media supplemented with phosphate. It has been shown that rat 1-cell embryos failed to reach the blastocyst stage in phosphate-supplemented KSOM-R, rather they were blocked at the 2-cell stage (Nakamura et al. 2016).

Amino acids: The importance of amino acids in the development of rat embryos was shown in different studies (Biggers et al. 1997, Ohboshi et al. 1998, Leese et al. 2021). Ammonium which is released after metabolizing amino acids has been reported to inhibit embryonic development of rat embryos cultured in vitro (Gardner and Lane 1993). Ho et al. (1995) found positive effects of low amounts of amino acids on mouse embryos; they also emphasized that embryonic development can be affected by the ammonium amount which can be adjusted by the volume of the media and embryo number per drop. Adding an Eagle's essential and nonessential amino acids has a beneficial impact on rat embryo development as same as the other species (Miyoshi et al. 1995a, Summers and Biggers 2003, Men et al. 2023).

For modeling the rat oviductal fluid, which is rich in taurine in all periods from the estrus cycle to the first three days of gestation, and contains high amounts of glycine, glutamate and alanine, the KSOM-R medium was developed based on KSOM-AA (amino acid added potassium simplex optimized media) (Nakamura et al. 2016). Adding amino acids to the

culture media has been shown to enhance the in vitro development of rat embryos and increase the blastocyst rate (Nakamura et al. 2016).

Protein sources: It has been known that the protein sources in embryo culture such as FBS (Foetal Bovine Serum Albumin), L-glutamine and BSA (Bovine Serum Albumin) are beneficial for mammalian preimplantation embryos (Kuran et al. 2001); contrary to this, there are opinions for that these sources have not positive effect on blastocyst formation (Gómez and Diez 2000). However, R1ECM which contains glutamine has been reported to support the development of rat embryos from morulae to blastocyst stage substantially, and it can be said that glutamine is an important chemical for in vitro culture of rat embryos (Miyoshi et al. 1995a). FBS, due to phosphate and growth factors it contains, has a deleterious effect on rat embryos from 1-cell to morulae (Han and Niwa 2003).

BSA which contains albumin can be added in embryo culture media as a resource of amino acids, fatty acids, and a small amount of vitamin sources, used to ease embryo manipulations, and additionally, used to be bound toxic heavy metals such as zinc and copper in culture media (Bavister 1995, Kito et al. 2008). Albumin is known to have both beneficial and detrimental effects in mammalian embryo culture (Bavister 1995). BSA has been understood to promote in vitro embryonic development of many mammalian embryos in the first cleavage stages and it is often used in mouse embryo culture media (McKiernan and Bavister 1992, Han and Niwa 2003, Zhou et al. 2003). Although hamster embryos are well developed in protein-free culture medium, it has been reported that BSA should not be considered completely ineffective for mammalian preimplantation embryos (McKiernan and Bavister 1992). Zhou et al. (2003) however reported that BSA harms rat embryos after the 1-cell stage. In contrast, it has been shown that BSA stimulated blastocyst formation and cell numbers in 2-cell rat embryos (Kito et al. 2008).

Phenol red: Phenol red is used as a pH indicator in cell culture media. Nakamura et al. (2016) reported that phenol red prevents blastocyst development of rat embryos in KSOM-R, thereby it has an estrogenic-like effect (Berthois et al. 1986, Ernst et al. 1989).

Osmolarity

The osmolarity of culture media is as important as media contents for all cell and embryo cultures. It has been shown that the role of the osmolarity of culture media in rat embryos is important (Miyoshi et al. 1994). Changing osmolarity depending on the presence of glucose and amino acids has been reported as a substantial factor in embryonic development in rats (Miyoshi et al. 1994, Ohboshi et al. 1998).

Miyoshi et al. (1994, 1995a) arranged medium osmolarity by changing NaCl concentrations and adding sorbitol and investigated the effects of osmolarity on rat embryo development. They found that rat embryos were sensitive to osmolarity and the optimal medium osmolarity for rat embryos was 244-246 mOsm. Sperm penetration in in vitro fertilization of rat embryos has been successfully achieved by increasing the NaCl concentration of mR1ECM (Oh et al. 1998). This situation has shown that rat embryos in early preimplantation stages could be developed in high osmolarity culture media as distinct from other species (Agca 2019). However, although the mR1ECM medium has a low amount of NaCl and its osmolarity is 246 mOsm, it can be increased the rate of blastocyst formation (Miyoshi et al. 1995a). It has been reported that KSOM-R (260 mOsm) was a supporting media for rat embryos, although it has a low osmolarity in comparison with embryo culture media used in other species (Nakamura et al. 2016, Men et al. 2020, Men et al. 2023). Considering all of these, it is possible to say that rat embryos have a different metabolism from other species (Popova et al. 2011).

The evaluation of rat embryo culture media success

Many researchers prefer to use mice in embryologic studies because mouse embryos are well known, and culture systems for mouse embryos successfully work. On the other hand, although rat, hamster, and rabbit embryos do not have improved embryo culture systems, using these animals in research is important for understanding the development of human preimplantation embryos (Seshagiri and Vani 2019). For this reason, it is very important to examine and develop the media used in the in vitro culture of rat embryos from past to present.

Kishi et al. (1991) have been reported that rat embryos cultured in HECM-1 were developed from zygote to blastocyst stage, but the blastocyst rate was low (9.9%). The more glucose-added HECM-1, which has low osmolarity by reducing the NaCl concentration and excluding amino acids from the medium, enhanced the development of rat embryos; after these changes, the rearranged medium was called mHECM-1 (Miyoshi et al. 1994). However, blastocyst formation (Table 1) in mHECM-1 has been shown that it does not better than mR1ECM or KSOM-R. Therefore, although it is not the first choice, mHECM-1 use as a culture medium may be preferred (Miyoshi et al. 1994, Miyoshi et al. 1995a, Nakamura et al. 2016).

Table 1. The development of rat embryos cultured in mHECM-1 medium.

References	Rat Strain	% zygote development to		
		≥2- cell	≥ 4- cell	Blastocyst
Miyoshi et al. 1994	Wistar	100	98	61
Matsumoto and Sugawara 1995	Wistar	94.4	93	71

Both in vitro fertilized or cultured rat zygotes cultured in mKRB (Table 2) have been reported to be blocked in the 2- or 4-cell stage (Toyoda and Chang 1974, Kishi et al. 1991). Moreover, in vivo developed rat embryos (obtained in 2- and 4-cell stages) could not arrive beyond the 4-cell stage when cultured in mKRB medium (Kishi et al. 1991). Rat 1-cell embryos obtained in vivo and developed in vitro have been reported that embryonic development was affected positively by transferring them to mR1ECM medium after being precultured with mKRB (Miyoshi et al. 1997). In similar research, short-term cultured rat embryos in mKRB before being cultured in mR1ECM have found that the blastocyst formation rate was higher than cultured only in mR1ECM (Kaneko et al. 2009). Therefore, it is possible to say using only mKRB medium as a culture media is not enough to develop rat embryos; however, if it is combined with other culture media, it can contribute to the development of rat embryos.

mR1ECM which is the most successful culture media than others has been reported as the only option to develop rat embryos for a long time (Nakamura et al. 2016), and is still used as one of the first choices as a culture medium due to obtaining very high blastocyst rates (Iannacone et al. 2001, Men et al. 2020). Although the number of live pups from the rat preimplantation embryos after in vitro culture in mR1ECM has been low (Miyoshi et al. 1995a, Kato et al. 2004, Nakamura et al. 2016), the rat preimplantation embryos have been developing in the mR1ECM medium successfully (Table 3).

KSOM medium which has high success rates in mouse embryo culture has been promising in developing rat embryos due to it can be editable for many species (Nakamura et al., 2016). The blastocyst

Table 2. The development of rat embryos cultured in mKRB medium and the collection time of embryos.

References	Rat Strain	Collection time of embryos	% embryos development to		
			≥2- cell	≥ 4- cell	Blastocyst
Kishi et al. 1991	Wistar	1-cell	76.2	4.1	0
Kaneko et al. 2009	Wistar	1-cell	90	42	28
Kishi et al. 1991	Wistar	2-cell	-	0	0
Kishi et al. 1991	Wistar	4-cell	-	0	0

Table 3. The development of rat 1-cell embryos cultured in mR1ECM medium.

References	Rat Strain	% zygote development to		
		≥ 2- cell	≥ 4- cell	Blastocyst
Miyoshi et al. 1995b	Wistar	100	73	58
Oh et al. 1998	Wistar	100	94.6	84.3
Han and Niwa 2003	Wistar	100	98	92
Iannaccone et al. 2001	Wistar Furth	56	35	0
Iannaccone et al. 2001	Lewis	13	13	9
Iannaccone et al. 2001	F344	72	42	12
Iannaccone et al. 2001	PVG	88	31	6
Kato et al. 2004	(SD x DA) x Wistar	99	41	33
Iannaccone et al. 2001	Sprague Dawley	96	94	76
Men et al. 2020	Sprague Dawley	95.9	61	18

Table 4. The development of 1- cell rat embryos cultured in KSOM-R medium.

References	Rat Strain	% zygote development to		
		≥2- cell	≥ 4- cell	Blastocyst
Nakamura et al. 2016	Wistar	~60	~85	~50
Men et al. 2020	Sprague Dawley	100	18	46

rates were found higher when 1-cell rat embryos (obtained 10-12 h after the ovulation) were cultured firstly in KSOM (for 18 h) and after in mR1ECM medium (Miyoshi et al., 1995b) than when cultured only in mR1ECM medium (Zhou et al. 2003). KSOM does not contain amino acids; KSOM-AA was developed after the importance of amino acids in the development of preimplantation embryos was understood. Moreover, KSOM-R which is based on KSOM-AA was designed by Nakamura et al. (2016) for rat embryos. In studies using mR1ECM and KSOM-R (Table 4) media, it has been shown both culture media promote the development of rat embryos cultured in vitro (Nakamura et al. 2016, Men et al. 2020, Men et al. 2023). However, the first research that compared these two culture media presented blastocyst rates of KSOM-R was better than mR1ECM (Men et al. 2020).

Conclusion

Rat embryo culture is not yet well understood, and the culture media need to improve. It can be possible to conclude that all present media for rat embryo culture are not optimal yet or that use of any of these media is not optimal for every strain or every research.

Since rat embryo culture media has been developed from the mouse embryo culture media, they all have similar contents. However, when compared with mouse embryo culture media, rat embryo culture media must have critical differences. Although these two species have similar genetic backgrounds, the present review shows that the effects of some of the culture media components on rat embryos are different from mouse embryos. Understanding these alterations is important to improve the current rat embryo culture media. Doing more investigations that compare mouse and rat embryos may help to understand rat embryos' needs in vitro.

Strain difference affects the success of culture media on the development of rat embryos in vitro; thereby, research with different rat strains may help to achieve optimal culture media or to understand which media is better for which strain.

mR1ECM medium has been successful in developing rat embryos from the 1-cell stage to the blastocyst stage, however, embryos developed in mR1ECM have a low capability of development in vivo and survival after embryo transfer. The in vitro development capability of culture media should support the subsequent in vivo development capability too. Therefore, it is possible to say that the mR1ECM medium still needs to be improved. Furthermore, although the results show that KSOM-R is successful in the development of rat embryos and their survival ability after embryo transfer, it needs more investigation for different strains.

References

Agca Y. (2019). *In vitro culture of rat preimplantation embryos. In J. Herrick (Ed).* Comparative Embryo Culture. Methods in Molecular Biology. vol 2006, 33-43, Humana, New York, NY.

Bavister, B. D. (1995). Culture of preimplantation embryos: facts and artifacts. *Human reproduction update, 1*(2), 91-148.

Berthois, Y, Katzenellenbogen, J. A, & Katzenellenbogen, B. S. (1986). Phenol red in tissue culture media is a weak estrogen: implications concerning the study of estrogen-responsive cells in culture. Proceedings of the *National Academy of Sciences, 83*(8), 2496-2500.

Biggers, J. D, Summers, M. C, & McGinnis, L. K. (1997). Polyvinyl alcohol and amino acids as substitutes for bovine serum albumin in culture media for mouse preimplantation embryos. *Human Reproduction Update, 3*(2), 125-135.

Brison, D. R, & Leese, H. J. (1991). Energy metabolism in late preimplantation rat embryos. *Reproduction, 93* (1), 245-251.

Ernst, M., Schmid, C., & Froesch, E. R. (1989). Phenol red mimics biological actions of estradiol: enhancement of osteoblast proliferation in vitro and of type I collagen gene expression in bone and uterus of rats in vivo. *Journal of steroid biochemistry, 33*(5), 907-914.

Gardner, D. K., & Lane, M. (1993). Amino acids and ammonium regulate mouse embryo development in culture. *Biology of Reproduction, 48*(2), 377-385.

Gómez, E, & Diez, C. (2000). Effects of glucose and protein sources on bovine embryo development in vitro. *Animal Reproduction Science, 58*(1-2), 23-37.

Han, M. S., & Niwa, K. (2003). Effects of BSA and fetal bovine serum in culture medium on development of rat embryos. Journal of Reproduction *and Development, 49*(3), 235-242.

Hickman, D. L., Johnson, J., Vemulapalli, T. H., Crisler, J. R., & Shepherd, R. (2017). *Commonly used animal models. In M. A Suckow, K.L. Stewart (Ed)*. Principles of animal research for graduate and undergraduate students, 117.

Ho, Y., Wigglesworth, K., Eppig, J. J., & Schultz, R. M. (1995). Preimplantation development of mouse embryos in KSOM: augmentation by amino acids and analysis of gene expression. Molecular *reproduction and development, 41*(2), 232-238.

Iannaccone, P., & Galat, V. (2014). *Production of transgenic rats. In C.A. Pinkert (Ed*). Transgenic animal technology 3th ed. (pp. 251-273). Elsevier.

Iannaccone, P., Taborn, G., & Garton, R. (2001). Preimplantation and postimplantation development of rat embryos cloned with cumulus cells and fibroblasts. *Zygote, 9*(2), 135-143.

Kaneko, T., Kimura, S., & Nakagata, N. (2009). Importance of primary culture conditions for the development of rat ICSI embryos and long-term preservation of freeze-dried sperm. *Cryobiology, 58* (3), 293-297.

Kato, M., Ishikawa, A., Hochi, S., & Hirabayashi, M. (2004). Donor and recipient rat strains affect full-term development of one-cell zygotes cultured to morulae/blastocysts. *Journal of Reproduction and Development, 50*(2), 191-195.

Kishi, J., Noda, Y., Narimoto, K., Umaoka, Y., & Mori, T. (1991). Block to development in cultured rat 1-cell embryos is overcome using medium *HECM*-1. *Human Reproduction, 6*(10), 1445-1448.

Kito, S., Kaneko, Y., Yano, H., Tateno, S., & Ohta, Y. (2008). Developmental responses of 2-cell embryos to oxygen tension and bovine serum albumin in Wistar rats. *Experimental Animals, 57*(2), 123-128.

Kuran, M., Robinson, J. J, Staines, M. E, & McEvoy, T. G. (2001). Development and de novo protein synthetic activity of bovine embryos produced in vitro in different culture systems. *Theriogenology, 55*(2), 593 -606.

Leese, H. J, McKeegan, P. J, & Sturmey, R. G. (2021). Amino acids and the early mammalian embryo: Origin, fate, function and life-long legacy. International *Journal of Environmental Research and Public Health, 18*(18), 9874.

Matsumoto, H., & Sugawara, S. (1995). Development of rat embryos at the 1-and 2-cell stage in modified HECM-1 medium after exposure to a medium that contained phosphate. *Journal of Reproduction and Development, 41*(4), 263-269.

Mayer, J. F., & Fritz, H. I. (1974). The culture of preimplantation rat embryos and the production of allophenic rats. *Reproduction, 39*(1), 1-9.

McKiernan, S. H, & Bavister, B. D. (1992). Different lots of bovine serum albumin inhibit or stimulate in vitro development of hamster embryos. *In Vitro Cellular & Developmental Biology*, 154-156.

Men, H., Amos-Landgraf, J. M., Bryda, E. C., & Franklin, C. L. (2023). KSOM-R supports both mouse and rat preimplantation embryo development in vitro. *Theriogenology, 198*, 69-74.

Men, H., Stone, B. J., & Bryda, E. C. (2020). Media optimization to promote rat embryonic development to the blastocyst stage in vitro. *Theriogenology, 151*, 81-85.

Miller, B. G, & Armstrong, D. T. (1981). Superovulatory doses of pregnant mare serum gonadotropin cause delayed implantation and infertility in immature rats. *Biology of Reproduction, 25*(2), 253-260.

Miyoshi, K. (2016). Development of a culture medium for rat 1-cell embryos. *Journal of Mammalian Ova Research, 33*(1), 11-16.

Miyoshi, K., & Niwa, K. (1997). Stage-specific requirement of phosphate for development of rat 1-cell embryos in a chemically defined medium. *Zygote, 5*(1), 67-73

Miyoshi, K, Abeydeera, L. R, Okuda, K., & Niwa, K. (1995a). Effects of osmolarity and amino acids in a chemically defined medium on development of rat one-cell embryos. *Reproduction, 103*(1), 27-32.

Miyoshi, K., Funahashi, H., Okuda, K., & Niwa, K. (1994). Development of rat one-cell embryos in a chemically defined medium: effects of glucose, phosphate and osmolarity. *Reproduction, 100*(1), 21-26.

Miyoshi, K., Kono, T., & Niwa, K. (1997). Stage-dependent development of rat 1-cell embryos in a chemically defined medium after fertilization in vivo and in vitro. *Biology of Reproduction, 56*(1), 180-185.

Miyoshi, K., Tanaka, N., & Niwa, K. (1995b). Penetration in vitro of naturally ovulated rat eggs and the development of eggs in a chemically defined medium. *Journal of Mammalian Ova Research, 12* (1), 35-39.

Nakamura, K., Morimoto, K., Shima, K., Yoshimura, Y., Kazuki, Y., Suzuki, O., Matsuda, J., & Ohbayashi, T. (2016). The effect of supplementation of amino acids and taurine to modified KSOM culture medium on rat embryo development. *Theriogenology, 86*(8), 2083-2090.

Oh, S. H, Miyoshi, K., & Funahashi, H. (1998). Rat oocytes fertilized in modified rat 1-cell embryo culture medium containing a high sodium chloride concentration and bovine serum albumin maintain developmental ability to the blastocyst stage. *Biology of Reproduction, 59*(4), 884-889.

Ohboshi, S., Fujihara, N., Yoshida, T., & Tomogane, H. (1998). Effects of glucose and amino acids on in-vitro development of rat preimplantation embryos, and morphological features of the developed blastocysts. *Animal Science and Technology, 69*, 637-645.

Popova, E., Bader, M., & Krivokharchenko, A. (2011). Effect of culture conditions on viability of mouse and rat embryos developed in vitro. *Genes, 2*(2), 332-344.

Schini, S. A, & Bavister, B. D. (1988). Two-cell block to development of cultured hamster embryos is caused by phosphate and glucose. *Biology of Reproduction, 39*(5), 1183-1192.

Seshagiri, P. B, & Vani, V. (2019). *Enabling hamster embryo culture system: development of preimplantation embryos. In J. Herrick (Ed)*. Comparative Embryo Culture: Methods and Protocols. vol 2006, 45-61, Humana, New York, NY.

Summers, M. C., & Biggers, J.D. (2003). Chemically defined media and the culture of mammalian preimplantation embryos: historical perspective and current issues. *Human Reproduction Update, 9*(6), 557-582.

Telford, N. A., Watson, A. J., & Schultz, G. A. (1990). Transition from maternal to embryonic control in early mammalian development: a comparison of several species. *Molecular Reproduction and Development, 26*(1), 90-100.

Toyoda, Y., & Chang, M. C. (1974). Fertilization of rat eggs in vitro by epididymal spermatozoa and the development of eggs following transfer. *Reproduction, 36*(1), 9-22.

Tsujii, H., & Nakamura, Y. (1999). Utilization of [U-14C] glucose by preimplantation rat embryo cultured in vitro. *Journal of Mammalian Ova Research, 16*(3), 94-97.

Vajta, G., Rienzi, L., Cobo A., & Yovich, J. (2010). Embryo culture: can we perform better than nature?. *Reproductive Biomedicine Online, 20*(4), 453-469.

Yamada, M, & Nishikimi, A. (1999). Inhibitory effect of phosphate on in vitro development of 2-cell rat embryos is overcome by a factor (s) in oviductal extracts. *FEBS Letters, 462*(1-2), 71-74.

Zhang, X., & Armstrong, D. T. (1990). Presence of amino acids and insulin in a chemically defined medium improves development of 8-cell rat embryos in vitro and subsequent implantation in vivo. *Biology of reproduction, 42*(4), 662-668.

Zhou, Y., Galat, V., Garton, R., Taborn, G., Niwa, K., & Iannaccone, P. (2003). Two-phase chemically defined culture system for preimplantation rat embryos. *Genesis, 36*(3), 129-133.