The effect of various concentration of Bovine Serum Albumin in Mice (Mus musculus) In vitro Embrio culture on the number of Blastocyst

by Epy Muhammad Luqman

Submission date: 20-Dec-2022 12:05PM (UTC+0800)
Submission ID: 1984847202
File name: ect_of_various_concentration_of_Bovine_Serum_Albumin_in_Mice.pdf (262.75K)
Word count: 3438
Character count: 17945

Eco. Env. & Cons. 27 (3) : 2021; pp. (1331-1336)
Copyright@ EM International
ISSN 0971–765X

The effect of various concentration of Bovine Serum Albumin in Mice (*Mus musculus*) *In vitro* Embrio culture on the number of Blastocyst

Ewik Fitri Pristya Ningrum[1], Mas'ud Hariadi[1], Sri Pantja Madyawati[1], Rimayanti[1], Tatik Hernawati[1], Widjiati[1], Viski Fitri Hendrawan[2] and Epy Muhammad Luqman[1*]

[1]Department of Veterinary Science Faculty of Veterinary Medicine, Universitas of Airlangga, Kampus C Unair Jl. Mulyorejo Surabaya, 60115, Indonesia
[2] Department of Animal Reproduction Faculty of Veterinary Medicine, Universitas Brawijaya, Malang 65151, Indonesia

(Received 17 April, 2021; Accepted 7 May, 2021)

ABSTRACT

This study aimed to determine the optimal concentration of Bovine Serum Albumin in increasing the amount of blastocyst. Oocytes were obtained from female mice Balb/C strains. Oocyte cells were collected and divided into 3 groups: T1 (MEM + BSA 3%), T2 (MEM + BSA 5%), and T3 (MEM + BSA 7%). Semen is collected from fertile male mice, then motile spermatozoa were put into a medium containing eggs. Media contained spermatozoa and eggs were stored in a 5% CO_2 incubator. Observations were made under an inverted microscope at 100x magnification. Embryos that developed to the blastocyst stage show with a clear cytoplasm, clear plasma membrane, no granularity, and clear pellucide zone.Based on statistical data analysis, there was a significant difference between the highest and lowest concentrations of $p<0.05$, with significant differences in the treatment group T1 ($54.00^{a} \pm 8.94$) and the treatment group T3 ($32.00^{b} \pm 10.95$). It can be concluded that the optimal concentration of Bovine Serum Albumin in supporting embryonic development is a concentration of 3%. The addition of Bovine Serum Albumin to the culture media was able to support the development of mouse embryos to the blastocyst stage.

Key words : *Food production, Blastocyst, Bovine serum albumin, In vitro culture*

Introduction

In vitro fertilization is fertilization of an oocyte cell and spermatozoa which is carried outside the mother's body. There are often occur obstacles in embryonic development. Barriers to embryonic development in*in vitro* culture are depended by the physiological conditions of the culture medium (Mirsageri, 2013). In *in vitro* culture, mice embryos need a medium that is able to support the development of mice embryos from zygotes to blastocyst (Khoirinaya, 2011). According to Jaswandi *et al.* (2001), that the success of embryo production is influenced by several factors including the quality of gametes (oocyte and sperm), culture medium, and culture system.

Culture medium containing nutrients was made nearly same to the composition of nutrients, electrolytes, and macromolecules that exist in the female reproductive tract (Widjiati *et al.*, 2012). The culture medium used for *in vitro* embryo production is often added to various components that can support embryonic development (Bavister *et al.*, 1995). The scientists added supplements to make the culture

Corresponding author's email: epy-m-l@fkh.unair.ac.id

medium more optimum. One protein that is often added to embryo culture medium is serum protein. Serum protein that is often used is Fetal Bovine Serum (FBS) or Bovine Serum Albumin (BSA) (Gordon, 2003).

Bovine Serum Albumin is a source of serum protein that contains lots of amino acids. The use of amino acids in culture medium can increase embryonic development (Lee *et al.*, 2004). Bovine Serum Albumin works by increasing the maturity of oocytes and also binds molecules including ions, free radicals, and steroids. This can prevent oxidation reactions, stabilize pH and osmolarity pressure (Lonergan *et al.*, 1999). In *in vitro* culture medium BSA captures toxic substances and inorganic ions and mediates CO_2 transport (Evecen *et al.*, 2004).

Research conducted by Pratiwi (2016) shows that the medium supplemented with BSA is better in supporting the development of sheep oocytes to be fertilized than groups without BSA. Research conducted by Asad *et al.* (2017) gave the result that BSA 2 mg/ml added as a supplement for the maturation of sheep oocytes and culture medium was able to increase the development of oocyte levels to the next stage. In stage 2 cell embryo culture, the addition of BSA which acts as a source of protein in the culture medium gives optimal results at the amount of 1 mg/ml with M 16 medium and 3 mg/ml with whitten's medium (Evecen *et al.*, 2004).

Based on the description above, it is necessary to do further research on the effect of various concentrations of Bovine Serum Albumin on the amount of mice blastocysts in *in vitro* culture.

Materials and Methods

Ethical approval: The use of animal models has passed ethical clearence from Animal Care and Use Committee(ACUC) Universitas Airlangga with registration number: 717-KE.

Materials and tools

This research was conducted at the *In Vitro* Fertilization Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya. The sample of this study was egg and spermatozoa of mice. The study was divided into 3 treatment groups 1 (T1), namely: Culture medium MEM added Bovine Serum Albumin 3%, treatment group 2 (T2): MEM culture medium added Bovine Serum Albumin 5%, treatment group 3 (T3): Medium MEM culture added 7% Bovine Serum Albumin.

The research materials used for this research were female strain Balb/C, Phosphate Buffer Saline (PBS), sterile aquadest, 0.9% physiological NaCl, Bovine Serum Albumin (BSA) (Sigma-Aldirch). 70% alcohol, Minimum Engle Medium or MEM (Sigma-Aldirch), mineral oil (Sigma®), Pregnant Mare Serum Gonadotropin (PMSG) (Intervet) and Human Chorionic Gonadotropin (HCG) (Chorulon®).

The tools used in this study include a test animal cage along with a cage lid, drinking bottles, scissors, tweezers, petridish disposable, disposable syringe, 5% CO_2 incubator, refrigerator, laminar air flow, infuse hose, inverted microscope, pasteur pipette, Bunsen burner, and tissue.

Superovulation Technique

Superovulation technique is performed by injection of 0.1 cc PMSG (Pregnant Mare Serum Gonadotropin) containing 5 IU intraperitonially. Forty-eight hours later the 0.1 cc injection of HCG (Chorionic Gonadotropin Hormone) containing 5 IU intraperitonially. After injection of HCG (Chorionic Gonadotropin Hormone), female mice are mated with a monomating male vasectomy. The next day or seventeen hours later a vaginal plug is examined, if a positive vaginal plug is considered to have occurred copulation (Widjiati *et al.*, 2012).

Egg Collection and Modification of Culture Medium

Female mice are sacrificed for egg collection by decapitation, then dissected and fallopian tubes were taken. The fallopian tubes are washed using PBS (Phosphate Buffer Saline), then transferred to petridish. Fallopian tubes are observed under an inverted microscope by tearing a fertilization sac, so that an oocyte is obtained. The egg is washed three times using MEM medium. The egg is then transferred to the MEM medium which has been added with Bovine Serum Albumin. The Bovine Serum Albumin added to the T1 group was 3%, the T2 group was 5%, and the T3 group was 7%, then incubated in a 5% CO_2 incubator at 37 °C while waiting for preparation of spermatozoa which will be used for in vitro fertilization.

Decapitation of male mice for sperm collection. Cauda epididymis was taken and washed on minimum eagle medium (MEM). Washed epididymis were then cut to release spermatozoa. The spermatozoa are then mixed into each of the existing ovum

cells for *in vitro* fertilization and incubated in a 5% CO_2 incubator at 37 °C.

In vitro Culture and Observation of Embryonic Development

After 4 hours the fertilized egg was washed to remove the remaining granulosa cells in the medium and transferred to a new culture medium for in vitro culture, each medium was given an additional BSA concentration of 3% in the T1 group, 5% T2 group and 7% in the T3 group. Embryo observations were carried out every day using an inverted microscope with a magnification of 40 times. The observed variable is the number of embryos that live or develop until the blastocyst stage.

Results and Discussion

In vitro culture technology is an attempt to resemble environmental conditions *in vivo* in producing embryos (Mirsageri, 2013). The application of biotechnology requires oocytes in large quantities, then the obtained oocytes are matured in vitro for the benefit of *in vitro* fertilization. Successful *in vitro* fertilization requires adequate readiness of the egg and sperm biologically and culture conditions that support the metabolic effectiveness of male and female gametes (Syaiful, 2011). The good quality of embryos is influenced by several factors such as the culture technique used, oocyte quality, culture medium, CO_2 levels, incubation temperature, and sterile environment need to be considered (Sunarno, 2006).

Culture medium in the development of mice embryos is often added to various components thatcan support embryonic development. The addition of Bovine Serum Albumin which is a source of protein can increase egg cell growth. Another function of Bovine Serum Albumin (BSA) is to maintain the pH in culture medium (Francis, 2010). Bovine Serum Albumin (BSA) also increases oocyte maturation, fertilization, and blastocyst formation. Bovine Serum Albumin (BSA) contains a protein with a molecular weight of 66.5 kDa which contributes to oocyte maturation (Asad *et al*., 2017). Research conducted by Asad *et al.* (2017) gave the result that BSA 2 mg/ml added as a supplement for the maturation of sheep oocytes and culture medium was able to increase the development of oocyte levels to the next stage. Bovine Serum Albumin functions is to bind ions, free radicals, and steroids (Pratiwi, 2016). Adequate capacity of serum albumin is able to inactivate toxic metabolism from free radical oxygen production and unite other components such as steroids, vitamins, and fatty acids (Luvoni, 2004).

Embryos were cultured in a 5% CO_2 incubator using the Medium Eagle Minimum (MEM) medium with the addition of Bovine Serum Albumin (BSA) to the culture medium with concentrations of 3%, 5%, and 7% respectively. The development of the embryo is observed under an inverted microscope at 100x magnification. Observations were made until the developing embryo reached the blastocyst stage.

Based on the data obtained, the results of the calculation of the percentage of embryos until blastocyst is showed in Table 1. The results of this study

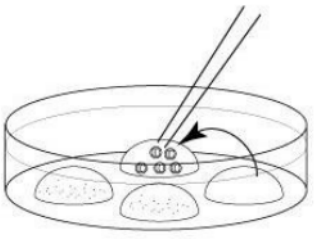

Fig. 1. Medium for washing (Center for Animal Resources and Development, 2016).

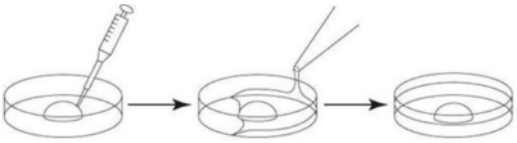

Fig. 2. Medium preparation (Center for Animal Resources and Development, 2016).

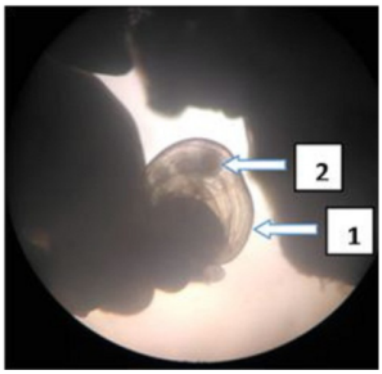

Fig. 3. Fertilization sac contained mice ovum. Description: 1=fertilization sac, 2=Ovum.

prove that the addition of BSA to in vitro culture medium is able to support the development of embryos reaching the blastocyst stage. The mean percentage of blastocysts in the treatment group supplemented with BSA with concentrations of 3%, 5%, and 7% respectively are 54.00 ± 8.944, 40.40 ± 7.335, and 32.00 ± 10.954. Groups T1 and T2 werenot significantly different (p>0.05). T1 and T3 groups were significantly different (p<0.05). T2 and T3 groups were not significantly different (p>0.05). Thus, it can be concluded that the highest mean percentage of blastocysts was in the T1 group with the addition of a 3% BSA.

Table 1. Average and standart deviation of blastocyst number each group of treatment

Group	Blastocyst number (Mean ± SD)
T1	54.00 [a] ± 8.944
T2	40.40 [ab] ± 7.335
T3	32.00 [b] ± 10.954

Note: Different superscripts in the same column show significant differences (p<0.05).
T1 = Medium MEM + *Bovine Serum Albumin* (BSA) 3%
T2 = Medium MEM + *Bovine Serum Albumin* (BSA) 5%
T3 = Medium MEM + *Bovine Serum Albumin* (BSA) 7%

Embryos that live up to the blastocyst stage can be identified based on their morphology, namely the presence of clear cytoplasm, clear plasma membrane, absence of granular-granular embryos, cytoplasm and pellucide zone are clearly visible. Dead blastocysts can be observed based on their morphology, namely the presence of granular embryos, the pellucide zone and the cytoplasm, which is not clearly visible, the size of the blastocysts decreased.

The results showed that, fertilized eggs were able to develop until the blastocyst stage with the addition of a 3% BSA concentration of 54%, a 5% BSA concentration of 40% and a 7% BSA concentration of 32%. It can be concluded that the addition of BSA can increase the percentage of oocyte life developing until the blastosis stage and the optimum results are obtained at a concentration of 3% BSA. The resultobtained equivalent to the study of Evecen (2004) which said that high concentrations of BSA in in vitro culture media can interfere with embryo development by affecting the pH of the medium.

High BSA concentration will affect the pH of the medium. In cell growth changes in pH in the medium will affect the structure of proteins (enzymes and transport systems) contained in cell membranes. Changes in the pH of the medium will affect the structure of the protein, which causes the protein to not be able to bind the compound that will be transported into the cell, causing the metabolism of cell growth to stop. A 3% BSA concentration is considered as the optimum dose to meet the needs of embryos in *in vitro* culture.

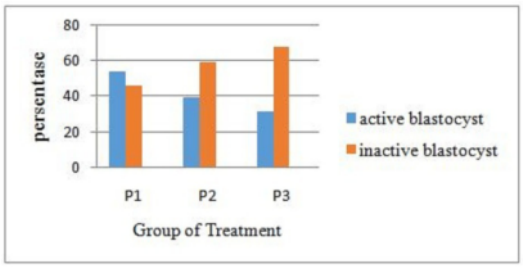

Fig. 4. Graphic of the effect of various concentration of bovine serum albumin toward the number of blastocyst

Uncertainty results from the addition of serum because serum contains not only protein, in addition to protein components (albumin), some hormones such as gonadotropins, steroids, and growth factors or cytokines. This is the reason why it is difficult to determine specific compounds that have a positive effect on embryo culture. Maybe the levels of the hormones or proteins contained in the serum of cows used are too high so they are toxic to the embryo itself. Another possibility that can occur is mixing of the factors contained in bovine serum so that the results get less than the maximum, so that one factor can be inhibiting for other factors (Fibrianto *et al.*, 2011). In this study the culture medium used was not replaced with a new culture medium or the nutrients given to the culture medium were the same as the beginning of administration. This is what causes the embryo is lack nutrition at the blastocyst stage. The greater the embryo, the higher the nutritional requirements needed. Addition of Bovine Serum Albumin concentration levels at various stages of embryonic development is needed further research.

Bovine Serum Albumin (BSA) is a serum albumin protein derived from cows. Molecular weight of Bovine Serum Albumin (BSA) 66.463 Da (= 66.5 kDa). Bovine Serum Albumin (BSA) is a single polypeptide chain consisting of about 583 amino

acid residues and does not contain carbohydrates. Albumin is the main protein found in serum with a percentage of around 50% - 60%. Albumin has a broad cell surface and able to binds molecules such as water, salt, free fatty acids, vitamins and hormones. Albumin acts as a nutrients transportation between molecules (Blake *et al.*, 2002). According to Francis (2010), albumin functions is to maintain pH, a carrier molecule by binding to ligands (fats, metal ions, amino acids), and as an antioxidant. In addition, the albumin molecule is able to interact with cells and promote cell growth.

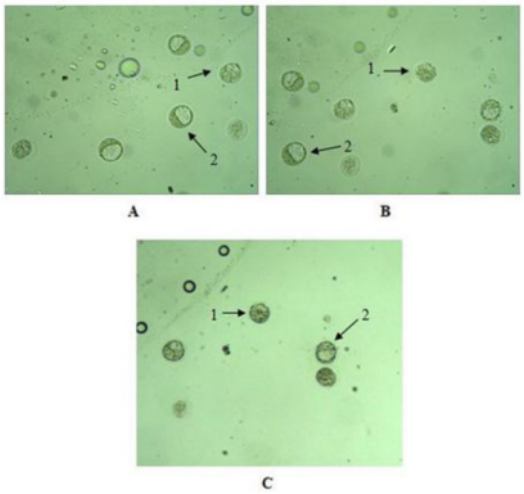

Fig. 5. Blastocyst in 100x magnification with an inverted microscope.A: groups T1, B: groups T2, C: group T3. Remarks: 1: initial blastosis, 2: expanded blastocysts.

Bovine Serum Albumin (BSA) increases maturation, fertilization, blastocyst formation, and maturation rates *in vitro* and has been widely used in the medium for sperm capacitation and acrosome reactions (Asad *et al.*, 2017). Bovine Serum Albumin (BSA) has a physiological role, functions as a pH stabilizer, regulates osmotic pressure, stabilizes membranes, contains amino acids, vitamins, fatty acids, hormones, growth factors, and is surfactant (Blake *et al.*, 2002). Bovine Serum Albumin (BSA) is generally added to culture media as a source of energy and protein for metabolic processes. The addition of Bovine Serum Albumin (BSA) can meet the needs of important components such as steroids, vitamins, fatty acids, and cholesterol, but also helps in the supply of ions and small molecules (Wrenzycki *et al.*, 2001).

Conclusion

Based on this study it can be concluded that, there are differences in the addition of various concentrations of Bovine Serum Albumin (BSA) to the amount of mice blastocyst in *in vitro* culture and the optimum amount of Bovine Serum Albumin (BSA) which appropriate in supporting the development of mice blasosis in *in vitro* culture is 3%. The addition of Bovine Serum Albumin (BSA) to the culture media was able to support the development of mice embryos to the blastocyst stage.

Acknowledgment

Thank you to the Director General of Research and Technology of Higher Education who helped support this research fund.

Conflict of Interests

The authors declare that they have no conflict of interests.

Authors' Contribution

EFPN designed the study, interpreted the data, and drafted the manuscript. MH, RSPM, VFH and R was involved in collection data and also contributed in manuscript preparation. TH, EML and W took part in preparing and critical checking of this manuscript.

References

Asad, L., Khandoker, M. A. M. Y. and Rahman, A. N.M. L. 2017. Effect of Bovine Serum Albumin on *In vitro* Maturation, *In vitro* Fertilization and Subsequent Development of Goat Embryos. *Int J Agric Innov Res.* 5 : 1096-1101.

Bavister, B. D. 1995. Culture of Preimplantation embryos: Facts and Artifacts. *Hum Reprod Update*. 1: 1481-1491.

Blake, D., Svalender, P., Jin, M., Silvers, C. and Hamberger, L. 2002. Protein Supplementation of Human IVF Culture Media. *J Assist Reprod Genet*. 19 : 3-10.

Center for Animal Resources and Development. Reproductive Engineering Technique in Mice. 2016 Kumamoto University, Japan.

Evecen, M., Serhat, P., Alkan, S. and Kamuran, I. 2004. The Effects of Various BSALevels in Different Mediaon Development in *In vitro* Culture of Mouse Embryos. Faculty of Veterinary Medicine. Istanbul University. Istanbul-Turkey.

Fibrianto, Y.H., Kusindarta, D.L. and Soebagyo, S. 2011.

Use of Inactivation Serum from Abattoirs in In vitro Fertilization Media. *Mediagama*. 2: 1-6.

Francis, G. L. 2010. Albumin and Mammalian Cell Culture: Implication For Biotechnology Applications. *Cytotechnology*. 62:1-26.

Gordon, I. 2003. Laboratory Production of Cattle Embryos 2nd Edition. Department of Animal Science and Production. University College Dublin Irish Republic.

Jaswandi, B. and Arief, Muhammad, A. S. 2001. *In Vitro* Maturation and Fertilization of Ovine Oocytes in a System with Absence of 5% CO_2. *Reprotech*. 1 : 19-22.

Khoirinaya, C. 2011. Viability of Mice Embryos (*Mus musculus albinus*) after cryopreservation with double vitrification at the zygote development stage and continued at the blastocyst stage. Bogor Agricultural Institute, Bogor.

Lee, E. S., Fukui, Y., Lee, B. C., Lim, J. M. and Hwang, W. S. 2004. Promoting Effect of Amino Acids Added to a Chemically Defined Medium on Blastocyst Formation and Blastomere Proliferation of Bovine Embryos Cultured *In Vitro*. *Anim Reprod Sci*. 84 : 257-267.

Lonergan, P. 1999. O'Kearney-Flynn M, BolandMP. Effect of Protein Supplementation and the Presence of an Antioxidant on the Development of Bovine Zygotes in Synthetic Oviduct Fluid Medium Under High or Low Oxygen Concentration. *Theriogenology*. 51 : 1565-1576.

Luvoni, G, C., Chigioni, S., Allievi, E. and Macis, D. 2004. Factors Involved *In vivo* and *In vitro* Maturation of Canine Oocytes. *Theriogenology*. 63 : 41-59.

Mirsageri, M. 2013. Development of Mice Embryos (*Mus musculus albinus*) in Modified CO_2 Incubators. Faculty of Veterinary Medicine. Bogor Agricultural Institute, Bogor.

Pratiwi, 2016. Maturation and Fertilization Level of Sheep Oocytes Maturated in Medium with Serum or Serum Substitute Components. Bogor Agricultural Institute, Bogor.

Syaiful, F.L., Saladin, R., Jaswandi, Udin, Z. 2011. Effect of Fertilization Time and Different Incubation Systems on the Level of Local Fertilization of Cows *In vitro*. Faculty of Animal Husbandry, University of Andalas. 13(1).

Sunarno, Embryo Aggregation in 8 Cell Cleavage Stage on KSOM Culture Medium to Produce Embryo Aggregation Results with High Viability Value: Study on Animal Model of Mice (*Mus musculus*). *Jurnal Sains dan Matematika*. 14 (4): 183-189.

Widjiati, S. E. and Pusporini, Arifin, M. Z. 2012. Comparison of Fertility Rates and Development Obstructions of Mice Embryos Cultured in M16 Medium and Human Tubal Fluid. *Jurnal Veteriner*. 13 (3) : 227-234.

Wrenzycki, C., Herman, D., Keskintepe, L., Martins, A., Sirisathien, S., Brackett, B. and Niemann, H. 2001. Effects of Culture System and Protein Supplementation on mRNA expression in Pre-implantation Bovine Embryos. *Hum Reprod*. 16 : 893-901.

The effect of various concentration of Bovine Serum Albumin in Mice (Mus musculus) In vitro Embrio culture on the number of Blastocyst

ORIGINALITY REPORT

16%	10%	13%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Gaia C. Luvoni, Sara Chigioni, Elisa Allievi, Debora Macis. "Factors involved in vivo and in vitro maturation of canine oocytes", Theriogenology, 2005 Publication	1%
2	nexusacademicpublishers.com Internet Source	1%
3	epubs.icar.org.in Internet Source	1%
4	journals.pan.pl Internet Source	1%
5	Waku, K.. "The effect of cadmium ions and cadmium-metallothionein on the activities of phospholipid-synthesizing enzymes of rat liver microsomes in vitro", Archives of Biochemistry and Biophysics, 19801001 Publication	1%
6	journals.tubitak.gov.tr Internet Source	1%

7	www.ijart.info Internet Source	1 %
8	bigdatainagriculture.com Internet Source	1 %
9	geomatejournal.com Internet Source	1 %
10	www.sigmaaldrich.com Internet Source	1 %
11	J. Dorval, V.S. Leblond, A. Hontela. "Oxidative stress and loss of cortisol secretion in adrenocortical cells of rainbow trout (Oncorhynchus mykiss) exposed in vitro to endosulfan, an organochlorine pesticide", Aquatic Toxicology, 2003 Publication	1 %
12	studentsrepo.um.edu.my Internet Source	1 %
13	Michael P. Mullen, Fuller W. Bazer, Guoyao Wu, Mervyn H. Parr, Alexander C. O. Evans, Mark A. Crowe, Michael G. Diskin. "Effects of systemic progesterone during the early luteal phase on the availabilities of amino acids and glucose in the bovine uterine lumen", Reproduction, Fertility and Development, 2014 Publication	1 %

14	Seong-beom Heo, Young-Sil Jeon, Seung Il Kim, Soo Hyun Kim, Ji-Heung Kim. "Bioinspired adhesive coating on PET film for antifouling surface modification", Macromolecular Research, 2013 Publication	<1 %
15	Methods in Molecular Biology, 2014. Publication	<1 %
16	Daniella A. J. Paula, Mônica R. F. Machado, Luis D. S. Murgas, Denílson Rabelo et al. "Toxicity of cryoprotectants on Prochilodus lineatus (Valenciennes, 1837) (curimba) embryos in an experimental incubator (Characiformes: Prochilodontidae)", Neotropical Ichthyology, 2014 Publication	<1 %
17	Young-Ho Choi, Charles C. Love, Dickson D. Varner, Linda B. Love, Katrin Hinrichs. "Effects of gas conditions, time of medium change, and ratio of medium to embryo on in vitro development of horse oocytes fertilized by intracytoplasmic sperm injection", Theriogenology, 2003 Publication	<1 %
18	dergipark.org.tr Internet Source	<1 %
19	docplayer.net Internet Source	

		<1%
20	patents.justia.com Internet Source	<1%
21	sherekashmir.informaticspublishing.com Internet Source	<1%
22	www.fasebj.org Internet Source	<1%
23	www.fmvz.unesp.br Internet Source	<1%
24	www.researcherslinks.com Internet Source	<1%
25	Barry D. Bavister. "Culture of preimplantation embryos: facts and artifacts", Human Reproduction Update, 1995 Publication	<1%
26	Hairong Su, Yulan Yang, Wanchun Lv, Xiaoli Li, Binxiu Zhao. "Bone marrow mesenchymal stem cell-derived exosomal microRNA-382 promotes osteogenesis in osteoblast via regulation of SLIT2", Research Square Platform LLC, 2022 Publication	<1%
27	Heidrun Gundlach, Meinhart H. Zenk. "Biological activity and biosynthesis of	<1%

pentacyclic oxylipins: The linoleic acid pathway", Phytochemistry, 1998
Publication

28	J. Biggers. "Polyvinyl alcohol and amino acids as substitutes for bovine serum albumin in culture media for mouse preimplantation embryos", Human Reproduction Update, 03/01/1997 Publication	<1 %
29	J. L. YANIZ. "In Vitro Development of Bovine Embryos Encapsulated in Sodium Alginate", Journal of Veterinary Medicine Series A, 10/2002 Publication	<1 %
30	Nasrin Sultana, Rafiqul Islam, Rekha Rani Das, Ziaul Haque, Kazi Rafiq, Md. Abu Hadi Noor Ali Khan. "Steroid growth promoter modified glucose profile and liver morphology in broiler by altering the localization and expression pattern of hepatic glucocorticoid receptors", Research in Veterinary Science, 2022 Publication	<1 %
31	core.ac.uk Internet Source	<1 %
32	A.T. Mukti, Y.G.P. Sari, G.S.R. Agusdinata, W.H. Satyantini, A.S. Mubarak, E.M. Luqman, Widjiati. "The effects of laserpuncture on gonadal maturity and sperm quality of male	<1 %

striped catfish (Pangasianodon hypophthalmus)", Theriogenology, 2020
Publication

33 D. Fischer Russell. "The impact of oocyte maturation media on early bovine embryonic development", Molecular Reproduction and Development, 10/2006 <1%
Publication

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	On		

The effect of various concentration of Bovine Serum Albumin in Mice (Mus musculus) In vitro Embrio culture on the number of Blastocyst

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6