Veterinary Research & Animal Husbandry

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Review Article | Journal of Veterinary Research and Animal Husbandry | Open Access

A Review of In Vitro Culture Systems in Bovine Reproductive Biotechnologies

Alper Kocyigit

Department of Reproduction and Artificial Insemination, University of Cumhuriyet, Sivas, Turkey

Received Date: February 23, 2016, **Accepted Date:** April 01, 2016, **Published Date:** April 11, 2016.

***Corresponding author:** *Alper Kocyigit, Department of Reproduction and Artificial Insemination, University of Cumhuriyet, Sivas, Turkey, Tel: 903-462-191-010; Fax: 903-462-191-110; E-mail: akocyigit@cumhuriyet.edu.tr*

Abstract

In vitro embryo production (IVP) is currently one of the most important biotechnologies in cattle breeding and husbandry. *In vitro* embryo technologies have enabled the production in large numbers of embryos of superior breeds in various livestock animals and allows for embryo transfer at low costs. Improvement of *in vitro* culture systems are important for production of embryos with high developmental competence that are used in embryo transfer programs. Even with the advancements of culture procedures, *in vitro* produced embryos, it usually shows low viability than developed *in vivo* counterparts. Furthermore, IVP embryos are more sensitive to cryoinjury than in vivo-derived embryos. with the number of bovine embryos transferred worldwide increasing annually, there is a greater need than ever to optimize conditions of embryo culture *in vitro* to maximize embryo quality, cryotolerance, and pregnancy rate. This review provides an overview of *in vitro* culture systems in bovine reproductive biotechnologies.

Keywords: Bovine; Embryo Production; Culture Systems

Introduction

In vitro embryo technologies have enabled the production in large numbers of embryos of superior breeds in various livestock animals and allows for embryo transfer at low costs [1] (Figure 1). They have also enabled the production of embryos for scientific research purposes from slaughtered or live animals. Although several decades of research have gone into *in vitro* culture conditions that promote the maximal embryo yield are yet to be standardized [3-5]. *In vitro* embryo culture systems depend on multiple parameters such as the composition of culture media and gases [6]. An efficient culture system for *in vitro* embryo development should be formulated to protect from intracellular stress and include all demands of the embryo thereby embryo can maintain viability. Various approaches have been employed to improve the culture systems of bovine embryos *in vitro*. The plurality of research toward improving embryo development *in vitro* has focused on culture composition, soluble chemicals and surrounding medium components [7,8]. Although with the advancements of culture procedures, *in vitro* produced embryos are usually shows low viability than developed *in vivo* counterparts. Furthermore, IVP embryos are more sensitive to cryoinjury than *in vivo*-derived embryos, such as shown by lower cell numbers, less compaction and a lower number of tight-junctions compared to embryos *in vivo* [9,10]. This review provides an overview of in vitro culture systems in bovine reproductive biotechnologies.

Culture Media

There are several treatises on the composition of embryo culture media and the role of specific medium components in supporting mammalian embryo development *in vitro*. It is essential to appreciate that the culture media are only one part of the overall culture system. Optimal culture media for embryo production appear to require a combination of various factors such as hormones, cytokines, growth factor, antioxidants, vitamins, enzymes and macromolecules [11-17]. The formulations of culture media are a key aspect of embryo culture [18] (Table 1).

The most common media used in those culture systems are SOF (synthetic oviduct fluid), KSOM, and CR1aa; nevertheless, other media, such as G1.1/G2.2, CR2aa, and TCM199, can also be used. To date there are different culture systems available for *in vitro* fertilized oocytes. They can be classified according to their formulation as follows: undefined, where serum or/and co-culture are used; semi-defined, where co-culture is omitted and serum is

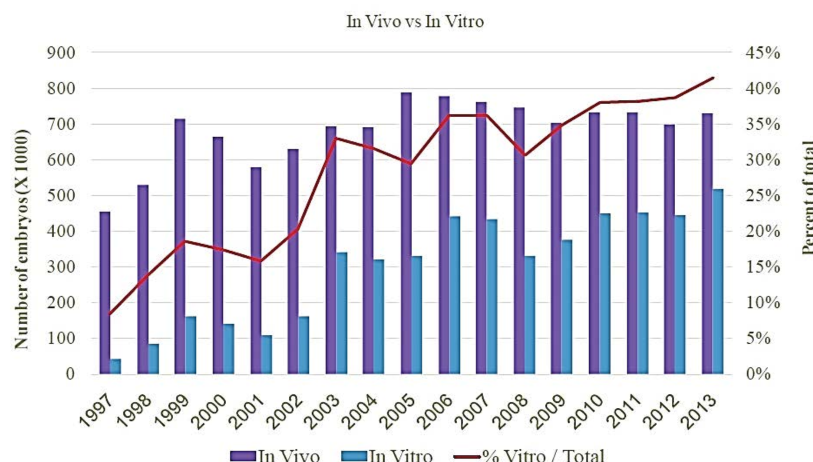

Figure 1: Worldwide *in vivo* and *in vitro* bovine embryo production between 1997 and 2013 [2].

Factor	Actions	Reference
Colony-stimulating factor 2	Blocked apoptosis in bovine embryos through regulatory actions on apoptosis genes.	[20]
Epidermal growth factor	Besides its role on proliferation, migration and differentiation, EGF is involved in reduction of apoptosis during preimplantation and placentation.	[18]
Fibroblast growth factor 2	FGF2 receptor activity were necessary for optimal development	[21]
Preimplantation factor	Involved in modulation of local immunity, promoting decidual proadhesion molecules and enhancement of trophoblast invasion.	[22]
Insulin, transferrin, sodium selenite	Insulin exerted its actions on glucose transport and AA uptake along with antioxidant protection from transferrin and selenium.	[18]
GH and IGF-I	In combination stimulated embryonic development.	[23,24]
IGF-II,FGF2, TGFβ1, CSF2	Results from the combination were similar to culture under serum conditions	[25]
LIF	LIF receptors have been detected in embryos of different species that is considered essential for blastocyst development such as hatching rate, cell count and cryotolerance	[26,27]
Activin A	Use of activin A shortened the lag phase and was also beneficial at later stages; however, more rapid rates of development were associated with higher rates of apoptosis.	[18]
Thyroid hormone	On the bovine embryo TH is involved in lowering apoptotic rates as well as improved hatching rates post thawing for cryopreserved embryos.	[28]

Table 1: Supplementation with embryotrophic factors towards improving defined media [19].

replaced by albumin; or fully defined, a protein-free system where albumin is replaced by macromolecules such as polyvinyl alcohol and polyvinyl pyrrolidone [29,30].

Culture Systems

Static systems

Due to changes in the embryo's requirements during growth, the use of culture media with formulations more similar to secretions found at different sites of the reproductive tissue. Sequential media have been developed to respond to the variable requirements of developing embryos.

Static or sequential systems require picking up and placing individual embryos several times. Embryos have been cultured on multiple configurations such as plastic polymers, petri dishes, and test tubes [31]. Additionally, this culture system seems not necessary since a single culture media support a full-term embryo development, and the extra manipulation of embryos associated with changing of media could be harmful [32,33].

Microdrops: The micro system involves the combination of a small incubation volume and a great volume around the embryo. In essence, the embryos create a micro-environment to retain embryotrophic growth factors, while still allowing small molecules to rapidly diffuse throughout the larger volume [30,34].

Microdrops have long been the approach used to restrict embryos to a small area to take advantage of the potential benefit of trophic factors [31]. Volumes of these drops typically range from; 10 to 50 µl, though some may be less and can be utilized with group as well as individual embryo culture. These low volumes and high embryo density culture approaches require high attention to medium properties because shifts in pH and osmolality are common and can have a profound impact on embryo development [31]. Microdrop dishes are now available to reduce this concern of embryo displacement, and they may be beneficial in some laboratories for embryo development by facilitating manipulation of the embryos during handling [31,35].

Microwells: This approach attempts to create a microenvironment in the immediate periphery of individual or small groups of embryos and offers a means of potentially increasing surface area point-of-contact and decreasing spacing between embryos [31]. Perhaps the most well-known microwell approach is the well-of-the-well (WOW) system [36]. The WOW system has been used successfully with embryos from a variety of species using small impressions of varying sizes and arrangements placed into the bottom of a dish [31,37].

Dynamic systems

In vivo, the developing embryo migrates from the oviduct to the uterine lumen where the fluid composition and gas atmosphere are likely different [32]. Due to changes in the embryo's requirements during migrates, the use of sequential culture media with various formulations. Therefore, static culture systems require picking up and placing individual embryos several times. Microfluidic systems, could eliminate most of this labor intensive handling and more importantly. Microfluidic systems allow a gradual alteration of culture media, offers a potential of automation, improved handling and possibly improves efficiencies through a reduction in environmental stress [31,38]. Embryos can be moved from one location to another, simulating the oviduct and uterine environments, by adjusting the fluid flow [30,38,39].

A dynamic culture system also provides a controlled opportunity to furnish embryos with a continually refreshed supply of new nutrients and removal of waste products [3].

Perfusion systems: The perfusion system is a concept for a continuous flow system for pre-implantation embryo culture, which has been tested in bovine embryo culture [34]. This system is oil-free with various advantages such as reduced exposure of

Concept	Principle	Media	Manipulation	Cost	References
Static	Same media for both PrC and PC	Drops (≈ 50 µl), wells (≈500 µl)	+++	+	[40]
Sequential	One media for PrC another one for PC	Drops (≈ 50 µl), wells (≈500 µl)	++++	++	[41,42]
Micro-culture system	Small incubation volume around the embryo	Drop of 0.06–0.24 µl	++	+++	[36,43]
Perfusion systems	Gradient changes of: Media components, Gas composition, temperature	Depending on the flow rates (30 to 70 µl min^{-1})	+	++++	[34]
Dynamic	A continued flow -microfluidic	≈ 0.125 µl of fluid medium	+	+++++	[39]

Table 2: Classification of mammalian embryo culture systems [30] (+ = low, ++++ = high; PrC = Pre-compaction; PC = Post-compaction).

media components to incubator and possibility of addition or removal of particular components at specific times during culture [30]. Its introduction in bovine embryo production techniques is still limited and might be due to its cost and availability of suitable equipment [30] (Table 2). Microfluidic perfusion technology is progressing rapidly as it seems that arduous is the only limiting factor when designing microfluidic devices [44].

Microfluidic systems can be proffer as micro scale perfusion systems that have the ability to operate over long periods with very little manipulation required [34,35]. We were able to produce an automated dynamic culture system for embryos that was not reliant on interconnections, which are historically known to make microfluidic systems complicated and for practical use. Further details of the system can be obtained in the original manuscript [45]. Come in view novel technologies such as microfluidics may provide further advances for producing high quality embryos in vitro before transfer to improve the possibility of high birth rates [19].

Conclusions

In vitro produced embryos are still less developmentally competence than their *in vivo* counterparts. There is an increased awareness of the stamina requirements at several stages of embryo development and the different requirements for achieving optimal rates of development. Although much can be learned from embryo culture systems that provide important insights, completely defined and optimized media is the goal [19]. Recent developments in culture systems and the potential to improve assisted reproductive technologies have been comprehensively reviewed by Smith et al [31]. Microfluidic dynamic systems and novel devices and platforms may offer a pathway toward improving IVP embryo viability within the laboratory in the future [31,46]. With the number of bovine embryos transferred worldwide increasing annually, there is a greater need than ever to optimize conditions of embryo culture systems to maximize viability, cryotolerance and pregnancy rate.

References

1. Thibier M. Data Retrieval Committee Annual Report, Year 2004. IETS Newsl. 2005;23:11-17.
2. Blondin P. Status of embryo production in the world. Anim Reprod. 2015;12(3):356-358.
3. Lane M1, Gardner DK, Hasler MJ, Hasler JF. Use of G1.2/G2.2 media for commercial bovine embryo culture: Equivalent development and pregnancy rates compared to co-culture. Theriogenology. 2003;60(3):407-19.
4. Hansen PJ, Block J. Towards an embryocentric world: the current and potential uses of embryo technologies in dairy production. Reprod Fertil Dev. 2004;16(1-2):1-14.
5. Cevik M, Kocyigit A, Sen U, Kuran M. Can Sequential Human Embryo Culture Media be Used in Bovine in vitro Embryo Culture? J F Vet Med Kafkas Uni. 2013; 20 (1):145-149.
6. Gardner DK, Lane M, Calderon I, Leeton J. Environment of the preimplantation human embryo in vivo: metabolite analysis of oviduct and uterine fluids and metabolism of cumulus cells. Fertil Steril. 1996;65(2):349-53.
7. Camargo LSA, Viana JHM, Sá WF, Ferreira AM, Ramos AA, Vale Filho VR. Factors influencing in vitro embryo production. Anim Reprod. 2006; 3(1):19-28.
8. Lonergan P, Fair T. The ART of studying early embryo development: progress and challenges in ruminant embryo culture. Theriogenology. 2014;81(1):49-55. doi: 10.1016/j.theriogenology.2013.09.021.
9. Thompson JG1. Comparison between in vivo-derived and in vitro-produced pre-elongation embryos from domestic ruminants. Reprod Fertil Dev. 1997;9(3):341-54.
10. Rizos D, Clemente M, Bermejo-Alvarez P, de La Fuente J, Lonergan P, Gutiérrez-Adán A. Consequences of in vitro culture conditions on embryo development and quality. Reprod Domest Anim. 2008;43 Suppl 4:44-50. doi: 10.1111/j.1439-0531.2008.01230.x.
11. Kim JH, Niwa K, Lim JM, Okuda K. Effects of phosphate, energy substrates, and amino acids on development of in vitro-matured, in vitrofertilized bovine oocytes in a chemically defined, protein-free culture medium. Biol Reprod. 1993;48(6):1320-5.
12. Rosenkrans CF Jr, First NL. Effect of free amino acids and vitamins on cleavage and developmental rate of bovine zygotes in vitro. J Anim Sci. 1994;72(2):434-7.
13. Lonergan P, Carolan C, Van Langendonckt A, Donnay I, Khatir H, Mermillod P. Role of epidermal growth factor in bovine oocyte maturation and preimplantation embryo development in vitro. Biol Reprod. 1996;54(6):1420-9.
14. Palasz AT, Thundathil J, Verrall RE, Mapletoft RJ. The effect of macromolecular supplementation on the surface tension of TCM-199 and the utilization of growth factors by bovine oocytes and embryos in culture. Anim Reprod Sci. 2000;58(3-4):229-40.
15. Rezaei N, Chian RC. Effects of essential and non-essential amino acids on in-vitro maturation, fertilization and development of immature bovine oocytes. Iran J Reprod Med. 2005; 3(1): 36–41.
16. Block J, Hansen PJ. Interaction between season and culture with insulin-like growth factor-1 on survival of in vitro-produced embryos following transfer to lactating dairy cows. Theriogenology. 2007;67(9):1518-29.
17. Duque P, Gómez E, Díaz E, Facal N, Hidalgo C, Díez C. Use of two replacements of serum during bovine embryo culture in vitro. Theriogenology. 2003;59(3-4):889-99.
18. Gordon I. Laboratory production of cattle embryos. Biotechnology in agriculture series. 2nd ed. Wallingford: CABI; 2003.
19. Absalón-Medina VA, Butler WR, Gilbert RO. Preimplantation embryo metabolism and culture systems: experience from domestic animals and clinical implications. J Assist Reprod Genet. 2014;31(4):393-409. doi: 10.1007/s10815-014-0179-2.
20. Loureiro B, Oliveira LJ, Favoreto MG, Hansen PJ. Colonystimulating factor 2 inhibits induction of apoptosis in the bovine preimplantation embryo. Am J Reprod Immunol. 2011;65(6):578-88. doi: 10.1111/j.1600-0897.2010.00953.x.
21. Fields SD, Hansen PJ, Ealy AD. Fibroblast growth factor requirements for in vitro development of bovine embryos. Theriogenology. 2011;75(8):1466-75. doi: 10.1016/j.theriogenology.2010.12.007.
22. Stamatkin CW, Roussev RG, Stout M, Absalon-Medina V, Ramu S, Goodman C, et al. PreImplantation Factor (PIF) correlates with early mammalian embryo development-bovine and murine models. Reprod Biol Endocrinol. 2011;9:63. doi: 10.1186/1477-7827-9-63.
23. Moreira F, Paula-Lopes FF, Hansen PJ, Badinga L, Thatcher WW. Effects of growth hormone and insulin-like growth factor-I on development of in vitro derived bovine embryos. Theriogenology. 2002;57(2):895-907.
24. Makarevich AV, Kubovičová E, Hegedušová Z, Pivko J, Louda F. Post-thaw culture in presence of insulin-like growth factor I improves the quality of cattle cryopreserved embryos. Zygote. 2012;20(2):97-102. doi: 10.1017/S0967199410000675.
25. Neira JA, Tainturier D, Peña MA, Martal J. Effect of the association of IGF-I, IGF-II, bFGF, TGF-beta1, GM-CSF, and LIF on the development of bovine embryos produced in vitro. Theriogenology. 2010;73(5):595-604. doi: 10.1016/j.theriogenology.2009.10.015.
26. Han YM, Lee ES, Mogoe T, Lee KK, Fukui Y. Effect of human leukemia inhibitory factor on in vitro development of IVF-derived bovine morulae and blastocysts, Theriogenology. 1995;44(4):507-16.
27. Kocyigit A, Cevik M. Effects of leukemia inhibitory factor and insulin-like growth factor-I on the cell allocation and cryotolerance of bovine blastocysts. Cryobiology. 2015;71(1):64-9. doi: 10.1016/j.cryobiol.2015.05.068.

28. Ashkar FA, Semple E, Schmidt CH, St John E, Bartlewski PM, King WA. Thyroid hormone supplementation improves bovine embryo development in vitro. Hum Reprod. 2010;25(2):334-44. doi: 10.1093/humrep/dep394.

29. Farin PW, Crosier AE, Farin CE. Influence of in vitro systems on embryo survival and fetal development in cattle. Theriogenology. 2001;55(1):151-70.

30. Feugang JM, Omar Camargo-Rodriguez, Erdogan M. Culture systems for bovine embryos. Livest Sci. 2009;121(2-3):141-149.

31. Smith GD, Takayama S, Swain JE. Rethinking in vitro embryo culture: new developments in culture platforms and potential to improve assisted reproductive technologies. Biol Reprod. 2012;86(3):62. doi: 10.1095/biolreprod.111.095778.

32. Fukui Y, Lee ES, Araki N. Effect of medium renewal during culture in two different culture systems on development to blastocysts from in vitro produced early bovine embryos. J Anim Sci. 1996;74(11):2752-8.

33. Gardner DK, Lane M. One-step versus two-step culture of mouse preimplantation embryos. Hum Reprod. 2006;21(7):1935-6; author reply 1936-9.

34. Thompson JG. Culture without the petri-dish. Theriogenology. 2007;67(1):16-20.

35. Rieger D, Schimmel T, Cohen J, Cecchi M. Comparison of GPS and standard dishes for embryo culture: set-up and observation times, and embryo development. Proceedings of the 14th World Congress on In Vitro Fertilization; 2007. p. 141.

36. Vajta G, Peura TT, Holm P, Páldi A, Greve T, Trounson AO, et al. New method for culture of zona-included or zona-free embryos: the well of the well (WOW) system. Mol Reprod Dev. 2000;55(3):256-64.

37. Vajta G, Korosi T, Du Y, Nakata K, Ieda S, Kuwayama M, et al. The well-of-the-well system: an efficient approach to improve embryo development. Reprod Biomed Online. 2008;17(1):73-81.

38. Glasgow IK, Zeringue HC, Beebe DJ, Choi SJ, Lyman JT, Chan NG, et al. Handling individual mammalian embryos using microfluidics. IEEE Trans Biomed Eng. 2001;48(5):570-8.

39. Wheeler MB, Clark SG, Beebe DJ. Developments in in vitro technologies for swine embryo production. Reprod Fertil Dev. 2004;16(1-2):15-25.

40. Summers MC, Biggers JD. Chemically defined media and the culture of mammalian preimplantation embryos: historical perspective and current issues. Hum Reprod Update. 2003;9(6):557-82.

41. Gardner DK. The road to single embryo transfer. Clin Embryol. 2004;7:16–26.

42. Gardner DK, Lane M. Ex vivo early embryo development and effects on gene expression and imprinting. Reprod Fertil Dev. 2005;17(3):361-70.

43. Taka M, Iwayama H, Fukui Y. Effect of the well of the well (WOW) system on in vitro culture for porcine embryos after intracytoplasmic sperm injection. J Reprod Dev. 2005;51(4):533-7.

44. Krisher RL, Wheeler MB. Towards the use of microfluidics for individual embryo culture. Reprod Fertil Dev. 2010;22(1):32-9. doi: 10.1071/RD09219.

45. Heo YS, Cabrera LM, Bormann CL, Shah CT, Takayama S, Smith GD. Dynamic microfunnel culture enhances mouse embryo development and pregnancy rates. Hum Reprod. 2010;25(3):613-22. doi: 10.1093/humrep/dep449.

46. Swain JE, Lai D, Takayama S, Smith GD. Thinking big by thinking small: application of microfluidic technology to improve ART. Lab Chip. 2013;13(7):1213-24. doi: 10.1039/c3lc41290c.

***Corresponding author:** Alper Kocyigit, Department of Reproduction and Artificial Insemination, University of Cumhuriyet, Sivas, Turkey, Tel: 903-462-191-010; Fax: 903-462-191-110; E-mail: akocyigit@cumhuriyet.edu.tr

Received Date: February 23, 2016, **Accepted Date:** April 01, 2016, **Published Date:** April 11, 2016.

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Citation: Kocyigit A (2016) A Review of In Vitro Culture Systems in Bovine Reproductive Biotechnologies. J Vet Res Ani Husb 1(1): 102.

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