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Popular Article

Medias used in *In Vitro* Embryo Production (IVEP): A Review

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Abstract

Assisted reproductive technologies (ARTs) can be any procedure, which involve the manipulation of reproductive cycles, gametes or embryos like Artificial Insemination, Multiple Ovulation Embryo Transfer (MOET), Semen sexing etc. *In Vitro* Embryo Production (IVEP) is one of the assisted reproductive technologies for faster propagation of superior germplasm in animals which includes Oocyte collection, *in vitro* maturation (IVM), *in vitro* fertilization (IVF) of oocytes with capacitated spermatozoa and *in vitro* culture (IVC) and Embryo transfer. For each step different medias are used in appropriate condition and concentration. Culture media are broadly divided into simple and complex. Simple media are usually bicarbonate-buffered systems and complex media contain, in addition to the basic components of simple media, amino acids, vitamins, purines, hormones, antioxidants, growth factors and other substances. It had been shown that monoculture medium system has the advantage of decreasing the number of manipulations and the length of time the embryo is out of the incubator when compared to sequential media for culture. Now a day, commercially available entire serum-free ready-to-use media are available, which suite for all the steps in IVEP. Long time effort of researchers has led to these successes in IVEP and still needs new research work in the field of IVEP for best outcomes.

Introduction

In Vitro Embryo Production (IVEP) is one of the assisted reproductive technologies for faster propagation of superior germplasm in animals. IVEP involves, collection of oocytes from either slaughterhouse ovaries or live animals through ultrasound guided transvaginal aspiration, oocyte *in vitro* maturation (IVM), *in vitro* fertilization (IVF) of oocytes with capacitated spermatozoa and *in vitro* culture (IVC) of presumptive zygotes (Gordon, 2004). In IVM, good quality oocytes are selected and kept in maturation media for 20-24 hrs in CO_2 incubator at 38.5°C, 5% CO2 and 90-95% relative humidity. After completion of IVM, oocytes are co-incubated with spermatozoa for up

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to 16 to 18 hrs while undergoing IVF (Gordon, 2004). After IVF, the fertilized oocytes are submitted to IVC media for 6-7 days until they reach the blastocyst stage. In general, 20% to 40% of the cultured presumptive zygotes will reach the blastocyst stage. After reaching the blastocyst stage, embryo transfer is performed following similar procedures as with *in vivo* blastocysts, or embryos are cryopreserved.

Medias in IVEP

For IVEP, different medias are used which have necessary elements for the development and maintenance of the oocytes, sperms, embryos and provides environment similar to the living body. Culture media are broadly divided into simple and complex.

Simple media are usually bicarbonate-buffered systems containing basic physiological saline with the addition of pyruvate, lactate and glucose; the main differences between the various forms of simple media lie in differences in their ion concentration and in the levels of the energy sources. The media are usually supplemented with serum or albumin with trace amounts of antibiotics (penicillin, streptomycin, gentamycin).

Complex media contain, in addition to the basic components of simple media, amino acids, vitamins, purines, hormones (FSH, LH, Prolactin, Growth hormone, Insulin, Estradiol, Melatonin etc.), antioxidants (glutathione, cystemaine, α-tocopherol, L-ascorbic acid, etc.), growth factors (IGF-1, IGF-2, EGF, bFGH, TGF-α and TGF-β_1) and other substances, mainly in the concentrations found in serum. Some of the complex media, including; Tissue Culture Media- 199 (TCM-199), Tyrode's Albumin Lactate Pyruvate (TALP) stocks, Synthetic Oviductal Fluid (SOF), and Minimum Essential Media (MEM) which have been used for oocytes maturation, fertilization and embryo development in mammals. The IVF culture also should be useful in providing sperm the needed movement and adaptation, which eventually leads to its union with the ova and then the beginning of embryonic development.

Commercially available medias

With the increasing implementation of IVEP of bovine embryos worldwide for commercial use, there is an increased focus on optimizing the yield of blastocysts. For this reason, in recent years, many companies have come up with commercially available medias having all essential components for oocyte, sperm and embryos development. These helps in performing all the procedures easily and with less contamination, in turn which helps in giving better results. Changes had been observed in the physiology and metabolism of the early developing bovine embryo, which gave increasing

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attention towards the use of sequential media for culture, each medium reflecting the changing requirements during development. But recently, in human as well as in bovine IVF the monoculture medium system is gaining popularity. The monoculture medium is supplemented with all the required compounds to sustain embryo development to the blastocyst stage, and is based on letting the embryo choose the nutrients and components needed for an optimum development during the entire culture period. It has been suggested that monoculture medium system is as efficient as the sequential medium system. Knowing that the embryos worst enemy is the fluctuations, in particularly, of pH and temperature (Swain, 2010), a monoculture medium system has the advantage of decreasing the number of manipulations and the length of time the embryo is out of the incubator.

Refined serum-free culture conditions, based on BSA supplementation, have been developed allowing for improved fetal development and calving (George *et al*., 2008), and in 2013 an entire serum-free ready-to-use media suite for all the steps, maturation, fertilization and culture, was made commercially available by IVF Bioscience, UK, combining synthetic serum replacements and BSA (Hyttel *et al*., 2019). Nielsen *et al.* (2015) concluded that the developmental rates and gene expression of *in vitro*-produced bovine blastocysts were affected by the use of different culture media. Increased blastocyst rates, apparently superior embryo quality, and more abundant gene expression were achieved when blastocysts were cultured in Bo-IVC culture media (IVF Biosciences) compared with SOF. Pryor *et al.* (2016) showed from his research that ETB (EmbryoTrans Biotech) media was superior to control media for percent viable, HBL (hatching blastocysts), and combined HBL/expanded BL (51.9, 23.9, 45.8% vs. 29.2, 5.8, 20.5, respectively). Also concluded that ETB media produced more high-quality embryos than control media under varying conditions experienced by commercial IVF companies.

Conclusion

In bovine IVEP, commercial 'ready to use' and 'serum-free' IVP media have contributed to a more stable production systems, as they reduce the batch-to-batch variability of a laboratory-made medium. Long time effort of researchers has led to this success in IVEP. Hence there is still need of new research work in the field IVEP for best outcomes.

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