

Improvements to *In Vitro* Culture Media for Use in Bovine IVF

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Abstract

In assisted reproduction of cattle the design and preparation of *in vitro* culture media has been instrumental in supporting the development of bovine oocytes and embryos. *In vitro* production (IVP) involves three main sequential steps: oocyte aspiration and *in vitro* maturation (IVM); *in vitro* fertilization (IVF); and *in vitro* culture (IVC) of early pre-implantation embryos. Research on IVC media has aimed to emulate the crucial elements that are present in the oviduct and uterus of female cattle by supplementing with protein and growth factors. As a result, IVP has proven a reliable methodology with an acceptable, if far from remarkable, blastocyst transfer rate of typically 40%. Moreover, the addition to media of defined extracts has gradually replaced that of fetal calf serum, leading to improved embryo viability and cryotolerance. Nevertheless, major advances in the composition of novel media that may facilitate a further increase of blastocyst rate have yet to be realized. Currently, there is considerable variation in the constituents of IVC media used in bovine assisted reproduction. Hence, significant innovations are required in order to not only achieve a greater success rate of IVP and to enhance the cryotolerance of bovine IVF-derived embryos but also to provide a defined IVC medium that may be recognized as a point of reference across the cattle breeding industry. This review examines the progress made to date in IVP to enhance embryo quality and points to future areas for investigation of a 'gold standard' IVC medium. This is a fully defined medium that supports consistently the attainment of a bovine IVP blastocyst rate similar to that achieved using a conventional medium supplemented with serum.

Keywords: *in vitro*; IVF; Culture; Oocyte; Embryo; Blastocyst; Oviduct; Bovine

Introduction

The emerging trend in bovine assisted reproduction in recent years is that *in vitro* production (IVP) is increasingly overtaking multi-ovulation embryo transfer as the technology of choice in several countries, including Brazil [1-3]. Moreover, Parrish [4] stated that bovine IVP has become a competitive alternative to multiple ovulation embryo transfer (MOET) and plays an important role in commercial settings. The reasons include the coupling of IVP with ovum pick-up (OPU) from live cows that enables aspiration of a large number of ovarian follicles from *Bos indicus* cattle oocytes without the stimulation of gonadotropin hormones [5]. In addition, highly transferable IVP embryos correlate with cattle possessing considerable numbers of ovarian follicles [6]. In addition, Pontes *et al.* [5] reported that the number of IVP embryos was greater than those attained by *in vivo* production over the same period of time. Moreover, Taylor-Robinson *et al.* [3] contended that IVP may increase genetic gain in a breeding herd, as OPU can be performed with young cattle [7]. Furthermore, there is no doubt that bovine *in vitro* fertilization (IVF) technology is an important research tool which may be used in order to understand early embryo development [8].

In terms of embryo production costs, Galli *et al.* [2] noted that for dairy cattle in Europe the cost of an IVP embryo is 1.5-2 times that of an *in vivo* embryo, leading to a modest demand; nevertheless, the costs of IVP in beef is still cheaper than MOET. Pre-selecting the gender of offspring through IVF with sex-sorted sperm can reduce the cost of embryo production because single sex-sorted semen straws can fertilize several cows in just one dish of the IVF system [9,10]. As a result, IVF technology that uses sex-sorted sperm is proven to be feasible under commercial conditions [10].

Despite such advantages over MOET, IVF remains a relatively inefficient process with blastocyst rates of around 40% [11,12]. This may be due to the fact that the support provided by the *in vitro* culture (IVC) system to oocytes subjected to IVP remains sub-optimal. IVP systems possibly lack physiological pathways and essential components that allow the development of bovine embryos [8].

Moreover, worldwide 90% of IVP embryos are transferred fresh, reflecting their lower cryotolerance [13]. Hence, modification of embryo culture media is necessary to improve the survival rates of IVP embryos [14]. Considerable attempts have been made to improve the yield of transferred blastocysts and their cryotolerance by supplementing IVC media with various growth factors and compounds thought to be stimulating [15-17]. Furthermore, research has focused attention on designing and establishing a novel defined culture medium that can replace serum under IVC conditions.

The purpose of this short review is to describe recent efforts to develop IVF systems that aim to enhance the blastocyst production and cryotolerance of IVP embryos. Significant improvements to and/or innovations in IVC conditions could make the use of IVF both more efficient and accessible and hence widespread in the cattle industry.

Variations of *In Vitro* Culture Media

An experimental approach has been successful in designing and generating various culture media based on an understanding of embryonic development in the female reproductive tract. Ovum-pick up technique allows the collection of bovine immature oocytes without the stimulation of gonadotropin hormones. These immature oocytes are then subjected to *in vitro* culture when they are influenced by various proteins and growth factors [18]. The most popular *in vitro* maturation (IVM) medium to use to support bovine oocyte *in vitro* maturation is tissue culture medium 199 (TCM199) supplemented with gonadotropin hormones and fetal calf serum (FCS). A study of the supplementation of TCM199 medium with tretinoin and lipid-core nanocapsules reported a blastocyst rate of 46.8% [19]. Based on TCM199 medium, Machado *et al.* [20] added fibroblast growth factor 17 alone or combination with bone morphogenetic protein 15 to IVM medium, which had the effect of increasing the expansion of cumulus cells, leading to an increase in blastocyst rates to around 50%. Bone morphogenetic protein 15 and fibroblast growth factor both regulate the function of cumulus cells and enhance embryo quality [20].

Typically, after the maturation of bovine oocytes in IVM for 22-24 hours, oocytes are subjected to fertilization. Two commonly used IVF media are Brackett-Oliphant medium and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)-buffered Tyrode's albumin lactate pyruvate (TALP) containing heparin. Tajik and Niwa [21] pointed out the synergistic effect of heparin and caffeine in IVF medium for spermatozoa capacitation. Monaco *et al.* [22], supplementing osteopontin in TALP medium, attained 56.4% blastocyst rates.

The success of embryo production is dependent not only on IVM and IVF media but also IVC medium. The ubiquitous IVC media currently used are CR1aa and modified synthetic oviduct fluid (SOF) supplemented with FCS and amino acids. The oviduct and uterus contain assorted specific regulatory elements, such as hormones, growth factors and cytokines, which when added to culture media are capable of enhancing the development of IVP pre-implantation embryos [14]. Neira *et al.* [16], testing the association of a wide range of governing factors (insulin-like growth factors I and II, basic fibroblast growth factor, transforming growth factors, granulocyte macrophage colony-stimulating factor, and leukemia inhibitory factor), indicated that supplementation of these components to IVC medium increased the yield of transferred blastocysts. In another study, Lim *et al.* [15] supplemented IVC medium with epidermal growth factor and reported a high blastocyst rate of 51.7% (108/261).

It is important to note that the supplementation of unusual components such as leptin [23], copper sulphate [24] and exogenous glutathione [25] to IVC has achieved better results. However, on some occasions the addition of factors like melatonin to IVM medium did not improve embryo production [26]. Another example is the addition to IVC medium of dexamethasone, which has the effect of raising the embryonic cell number but does not lessen the apoptosis rate of IVP embryos [27].

Furthermore, serum is a common supplement to IVC media, for which the concentration routinely added is 10% v/v [28,29]. FCS consists of 60-80 g protein (of which albumin accounts for 60%), growth factors, energy metabolites including glucose, amino acids and vitamins (Catt S, personal communication). Therefore, FCS is an important component that supports the maturation of oocytes and subsequent development of embryos [30]. In corroboration, FCS improves cell proliferation and assists blastocyst hatching [16]. Nevertheless, the presence of FCS in media increases the lipid content of IVP embryos [31,32], causing a detrimental effect on their cryosurvival. Thus, a reduction of serum level to 2% v/v in IVC medium is necessary to maintain blastocyst yield and embryo quality [33]. Moreover, supplementing IVC media with FCS can lead to inconsistent results between different IVP sessions [18], due to the intrinsically variable composition of FCS [16].

Development of Defined *In Vitro* Culture Media

The aim of preparing defined media is to minimize the variation in unknown components that occurs naturally in serum. This has the advantage of providing a simplified technique that enhances cryoresistance of embryos. This is due to avoiding the supplementation of biological components such as FCS and bovine serum albumin (BSA) that may increase the potential risks of contamination [16].

Bone morphogenetic protein 15 and fibroblast growth factor regulate the function of cumulus cells and increase embryo quality [20]. In spite of this, FCS is still often used in IVM medium, contrary to the rationale that defined media are beneficial for reasons of biosafety and identification of standardized constituents [34,35]. Evidence shows that use of defined media arrests the nuclear maturity of oocytes cultured for 24 hours [36].

Currently, a dependency on the addition of sera continues since an innovative medium that provides a complete substitute for IVM has yet to be introduced for use in bovine IVP. Previously, Holm *et al.* [30] attained a high percentage of blastocysts from defined IVC medium; however, they concluded that FCS is essential for IVM. Lee *et al.* [37] developed a defined IVC medium by supplementing with non-essential and essential amino acids, but the blastocyst rates in this study did not reach above 40%. In addition, Neira *et al.* [16] designed a fully defined culture medium with the addition of a range of recombinant growth factors and cytokines; however, the blastocyst rates (ranging from 20 to 48%) that resulted from using the defined medium were no greater than those from non-defined culture medium supplemented with FCS.

It may therefore be concluded that semi-defined *in vitro* media have been prepared successfully but that a fully defined *in vitro* medium remains to be formulated.

Cryopreservation of IVP Embryos

Commensurate with the advancement in cryopreservation technology, a modification to IVC media is critical in order to improve the survival rates of IVP embryos post cryopreservation [38]. Supplementation of silk protein sericin in the freezing medium but without serum results in similar survival and hatching rates of IVP embryos to those of freezing medium containing serum [35]. Addition of Albumax® 1 (lipid-rich BSA) to freezing medium also achieved a better survival of IVP blastocysts (87.3%) and calving rate (22.6%) compared to rates of survival (70.9%) and calving (10%) when using freezing medium containing fetal bovine serum [39]. Nevertheless, the freezing of IVP embryos represents a considerable challenge [5]. Vitrification offers a promising alternative cryopreservation technique for such sensitive cells [38,40]. For example, Sanches *et al.* [40] reported a high mean pregnancy rate of 48.8% after the transfer of vitrified embryos.

Indeed, the quality of IVP embryos is lower than that of *in vivo* counterparts [41], *in vivo* embryos showing less lipid accumulation, low apoptotic index and better cryotolerance than do IVP embryos [42]. Moreover, it is thought that the main difficulty encountered in freezing IVP embryos is due to excessive accumulation of intracytoplasmic lipid droplets [40-43]. The restoration of IVP embryos after cryopreservation is hampered as lipid droplets affect cellular repair [44]. Consequently, efforts to enhance the quality of IVP embryos prior to their cryopreservation have been made by modification of IVC media. It is evident that those IVP blastocysts considered to be of excellent quality, 'grade A', had a greater cryosurvival than those classed as 'grade B', of good quality (57.2% versus 41.7%) [45]. Consistent with this, supplementation of the naturally occurring antioxidant L-ergothioneine to IVC medium improves embryo quality and hence promotes an increase in the hatching rates of IVP embryos following cryopreservation [17].

The presence of serum in media is the main cause of increased lipid content of IVP embryos [31,43]. Thus, the main purpose of the improvement in the quality of IVP embryos before cryopreservation is to reduce or eliminate totally the need to use FCS in culture media.

Sudano *et al.* [32] found that for IVC a reduction of FCS to 2.5% v/v and addition of phenazine ethosulfate, thereby inhibiting fatty acid synthesis, improves the cryotolerance of IVP embryos. In addition, IVP bovine embryos cultured in this medium without FCS showed greater survival rates after cryopreservation than those cultured in medium with FCS [44]. For example, Gomez *et al.* [45] reported that IVP blastocysts cultured in SOF medium plus BSA without FCS showed a higher survival rate after vitrification than those cultured in SOF with FCS (56.6% and 35.2%, respectively). Furthermore, Block *et al.* [46] indicated that supplementation of hyaluronan to SOF medium improved the bovine IVP survival rate following vitrification.

Bovine IVP Using Sex-Sorted Sperm

IVF technology provides the fertilization efficiency of sex-sorted sperm [9]. Artificial insemination with sex-sorted sperm shows a lower rate of pregnancy loss when compared to IVP with sex-sorted sperm, suggesting that IVC media require improvement in order to mimic closely *in vivo* conditions [47]. Ghys *et al.* [48] showed that female IVP embryos were more apoptotic than their male counterparts. They also found that apoptosis was compounded when embryos were cultured in IVC with 5% v/v FCS compared to IVC containing BSA, insulin, transferrin and selenium. In fact, IVP systems for sex-sorted sperm have not been specialized for resultant embryos. However, Liu *et al.* [49] indicated that the quality of sex-sorted semen is lower than that of conventional semen. In addition, the reduced efficacy of sex-predetermined IVP is due to the low fertility of sperm processed by flow cytometry [10], as well as to individual variations in bull fertility [49-51]. Recently, Li *et al.* [52] have reported that isolation of motile sex-sorted sperm by use of a microfluidic sperm sorter resulted in a blastocyst rate of around 40%, which is similar to blastocyst yields achieved with non sex-sorted sperm.

Conclusion

In order to improve the generation of bovine IVP embryos many attempts have been made to develop a suitably supportive IVC medium by the addition of various factors. However, production of bovine embryos by IVF remains sub-optimal. The elimination of batch-to-batch variations in IVP by replacing sera with a novel defined IVC medium has not been fulfilled. To an extent reduction of the use of sera in preparing defined media enhances the cryotolerance of IVP embryos during the cooling and warming processes. Nonetheless, at present the IVP blastocyst yield that is achieved using defined IVC media is unsatisfactory; consequently, the

replacement of serum in media by synthetic extracts remains a practical difficulty. Cryopreservation of IVP embryos still poses a technical challenge, as their survival rates after cryopreservation are lower than those of *in vivo* counterparts. IVP with semen has to overcome the motility of sex-sorted sperm. These obstacles may be resolved by further investigation of IVC media and related *in vitro* preparatory techniques. This has the potential to achieve a significant increase in the efficacy of IVP, which, in turn, would lead to its more extensive application in assisted reproduction by the cattle industry.

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References

1. Camargo LS, Freitas C, de Sa WF, de Moraes Ferreira A, Serapiao RV, et al. (2010) Gestation length, birth weight and offspring gender ratio of *in vitro*-produced Gyr (*Bos indicus*) cattle embryos. *Anim Reprod Sci* 120: 10-5.
2. Galli C, Duchia R, Colleoni S, Lagutina I, Lazzari G (2014) Ovum pick up, intracytoplasmic sperm injection and somatic cell nuclear transfer in cattle, buffalo and horses: from the research laboratory to clinical practice. *Theriogenology* 81: 138-51.
3. Taylor-Robinson AW, Walton S, Swain DL, Walsh KB, Vajta G (2014) The potential for modification in cloning and vitrification technology to enhance genetic progress in beef cattle in Northern Australia. *Animal Reproduction Science* 148: 91-6.
4. Parrish JJ (2014) Bovine *in vitro* fertilization: *in vitro* oocyte maturation and sperm capacitation with heparin. *Theriogenology* 81: 67-73.
5. Pontes JH, Nonato-Junior I, Sanches BV, Ereno-Junior JC, Uvo S, et al. (2009) Comparison of embryo yield and pregnancy rate between *in vivo* and *in vitro* methods in the same Nelore (*Bos indicus*) donor cows. *Theriogenology* 71: 690-7.
6. Santos GM, Silva-Santos KC, Barreiros TR, Morotti F, Sanches BV, et al. (2016) High numbers of antral follicles are positively associated with *in vitro* embryo production but not the conception rate for FTAI in Nelore cattle. *Anim Reprod Sci* 165: 17-21.
7. Kruip TA, Boni R, Wurth YA, Roelofsen MW, Pieterse MC (1994) Potential use of ovum pick-up for embryo production and breeding in cattle. *Theriogenology* 42: 675-84.
8. Besenfelder U, Havlicek V, Kuzmany A, Brem G (2010) Endoscopic approaches to manage *in vitro* and *in vivo* embryo development: use of the bovine oviduct. *Theriogenology* 73: 768-76.
9. Xu J, Chaubal SA, Du F (2009) Optimizing IVF with sexed sperm in cattle. *Theriogenology* 71: 39-47.
10. Morotti F, Sanches BV, Pontes JH, Basso AC, Siqueira ER, et al. (2014) Pregnancy rate and birth rate of calves from a large-scale IVF program using reverse-sorted semen in *Bos indicus*, *Bos indicus-taurus*, and *Bos taurus* cattle. *Theriogenology* 81: 696-701.
11. Kepkova KV, Vodicka P, Toralova T, Lopatarova M, Cech S, et al. (2011) Transcriptomic analysis of *in vivo* and *in vitro* produced bovine embryos revealed a developmental change in cullin 1 expression during maternal-to-embryonic transition. *Theriogenology* 75: 1582-95.
12. Pellegrino CA, Morotti F, Untura RM, Pontes JH, Pellegrino MF, et al. (2016) Use of sexed sorted semen for fixed-time artificial insemination or fixed-time embryo transfer of *in vitro*-produced embryos in cattle. *Theriogenology* doi: 10.1016/j.theriogenology.2016.03.010.
13. Stroud B (2011) The year 2010 worldwide statistics of embryo transfer in domestic farm animals. In: IETS 2011 Statistics and data retrieval committee report. *Embryo Transfer Newsletter* 29: 14-23.
14. Block J, Hansen PJ, Loureiro B, Bonilla L (2011) Improving post-transfer survival of bovine embryos produced *in vitro*: actions of insulin-like growth factor-1, colony stimulating factor-2 and hyaluronan. *Theriogenology* 76: 1602-9.
15. Lim KT, Jang G, Ko KH, Lee WW, Park HJ, et al. (2007) Improved *in vitro* bovine embryo development and increased efficiency in producing viable calves using defined media. *Theriogenology* 67: 293-302.
16. Neira JA, Tainturier D, Peña MA, Martal J (2010) Effect of the association of IGF-I, IGF-II, bFGF, TGF- β 1, GM-CSF, and LIF on the development of bovine embryos produced *in vitro*. *Theriogenology* 73: 595-604.
17. Zullo G, Albergo G, Neglia G, De Canditiis C, Bifulco G, et al. (2015) L-ergothioneine supplementation during culture improves quality of bovine *in vitro*-produced embryos. *Theriogenology* 85: 688-97.
18. Rätym M, Ketoja E, Pitkänen T, Ahola V, Kananen K, et al. (2011) *In vitro* maturation supplements affect developmental competence of bovine cumulus-oocyte complexes and embryo quality after vitrification. *Cryobiology* 63: 245-55.
19. Lucas CG, Remião MH, Komninou ER, Domingues WB, Haas C, et al. (2015) Tretinoin-loaded lipid-core nanocapsules decrease reactive oxygen species levels and improve bovine embryonic development during *in vitro* oocyte maturation. *Reprod Toxicol* 58: 131-9.
20. Machado MF, Caixeta ES, Sudiman J, Gilchrist RB, Thompson JG, et al. (2015) Fibroblast growth factor 17 and bone morphogenetic protein 15 enhance cumulus expansion and improve quality of *in vitro*-produced embryos in cattle. *Theriogenology* 84: 390-8.
21. Tajik P, Niwa K (1998) Effects of caffeine and/or heparin in a chemically defined medium with or without glucose on *in vitro* penetration of bovine oocytes and their subsequent development. *Theriogenology* 49: 771-7.
22. Monaco E, Gasparini B, Boccia L, De Rosa A, Attanasio L, et al. (2009) Effect of osteopontin (OPN) on *in vitro* embryo development in cattle. *Theriogenology* 71: 450-7.
23. Córdova B, Morató R, Frutos C, Bermejo-Álvarez P, Paramio T, et al. (2011) Effect of leptin during *in vitro* maturation of prepubertal calf oocytes: embryonic development and relative mRNA abundances of genes involved in apoptosis and oocyte competence. *Theriogenology* 76: 1706-15.
24. Picco SJ, Rosa DE, Anchordoquy JP, Anchordoquy JM, Seoan A, et al. (2012) Effects of copper sulphate concentrations during *in vitro* maturation of bovine oocytes. *Theriogenology* 77: 373-81.
25. Sun WJ, Pang YW, Liu Y, Hao HS, Zhao XM, et al. (2015) Exogenous glutathione supplementation in culture medium improves the bovine embryo development after *in vitro* fertilization. *Theriogenology* 84: 716-23.
26. Takada L, Junior AM, Mingoti GZ, Balieiro JCC, Cipolla-Neto J, et al. (2012) Effect of melatonin on DNA damage of bovine cumulus cells during *in vitro* maturation (IVM) and on *in vitro* embryo development. *Res Vet Sci* 92: 124-7.

27. Santana PP, Carvalho CM, da Costa NN, Silva TV, Ramos PC, et al. (2014) Effect of dexamethasone on development of *in vitro*-produced bovine embryos. *Theriogenology* 82: 10-6.
28. Walters AH, Eyestone WE, Saacke RG, Pearson RE, Gwazdauskas FC (2005) Bovine embryo development after IVF with spermatozoa having abnormal morphology. *Theriogenology* 63: 1925-37.
29. Zhao S, Liu ZX, Gao H, Wu Y, Fang Y, et al. (2015) A three-dimensional culture system using alginate hydrogel prolongs hatched cattle embryo development *in vitro*. *Theriogenology* 84: 184-92.
30. Holm P, Booth PJ, Schmidt MH, Greve T, Callesen H (1999) High bovine blastocyst development in a static *in vitro* production system using SOFaa medium supplemented with sodium citrate and myo-inositol with or without serum-proteins. *Theriogenology* 62: 683-700.
31. Abe H, Yamashita S, Itoh T, Satoh T, Hoshi H (1999) Histochemical and ultrastructural evaluations of cytoplasmic lipid droplets in bovine embryos cultured in serum-free and serum-containing media. *Theriogenology* 51: 232.
32. Sudano MJ, Paschoal DM, Rascado Tda S, Magalhães LC, Crocomo LF, et al. (2011) Lipid content and apoptosis of *in vitro*-produced bovine embryos as determinants of susceptibility to vitrification. *Theriogenology* 75: 1211-20.
33. Leivas FG, Brum DS, Fialho SS, Saliba WP, Alvim MT, et al. (2011) Fetal calf serum enhances *in vitro* production of *Bos taurus indicus* embryos. *Theriogenology* 75: 429-33.
34. Saadeldin IM, Kim B, Lee B, Jang G (2011) Effect of different culture media on the temporal gene expression in the bovine developing embryos. *Theriogenology* 75: 995-1004.
35. Isobe O, Ikebata Y, Onitsuka T, Do LTK, Sato Y, et al. (2013) Cryopreservation for bovine embryos in serum-free freezing medium containing silk protein sericin. *Cryobiology* 67: 184-7.
36. Oliveira e Silva I, Vasconcelos RB, Caetano JV, Gulart LV, Camargo LS, et al. (2011) Induction of reversible meiosis arrest of bovine oocytes using a two-step procedure under defined and nondefined conditions. *Theriogenology* 75: 1115-24.
37. Lee ES, Fukui Y, Lee BC, Lim JM, Hwang WS (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: 257-67.
38. Nedambale TL, Dinnyes A, Groen W, Dobrinsky JR, Tian XC, et al. (2004) Comparison of *in vitro* fertilized bovine embryos cultured in KSOM or SOF and cryopreserved by slow freezing or vitrification. *Theriogenology* 62: 437-49.
39. Lim KT, Jang G, Ko KH, Lee WW, Park HJ, et al. (2008) Improved cryopreservation of bovine preimplantation embryos cultured in chemically defined medium. *Anim Reprod Sci* 103: 239-48.
40. Sanches BV, Marinho LS, Filho BD, Pontes JH, Basso AC, et al. (2013) Cryosurvival and pregnancy rates after exposure of IVF-derived *Bos indicus* embryos to forkolin before vitrification. *Theriogenology* 80: 372-7.
41. Pereira RM, Carvalhais I, Pimenta J, Baptista MC, Vasques MI, et al. (2008) Biopsied and vitrified bovine embryos viability is improved by trans10, cis12 conjugated linoleic acid supplementation during *in vitro* embryo culture. *Anim Reprod Sci* 106: 322-32.
42. Pryor JH, Looney CR, Romo S, Kraemer DC, Long CR (2011) Cryopreservation of *in vitro* produced bovine embryos: effects of lipid segregation and post-thaw laser assisted hatching. *Theriogenology* 75: 24-33.
43. Cagnone G, Sirard MA (2014) The impact of exposure to serum lipids during *in vitro* culture on the transcriptome of bovine blastocysts. *Theriogenology* 81: 712-22.
44. Mucci N, Aller J, Kaiser GG, Hozbor F, Cabodevila J, et al. (2006) Effect of estrous cow serum during bovine embryo culture on blastocyst development and cryotolerance after slow freezing or vitrification. *Theriogenology* 65: 1551-62.
45. Gómez E, Rodríguez A, Muñoz M, Caamaño JN, Hidalgo CO, et al. (2008) Serum free embryo culture medium improves *in vitro* survival of bovine blastocysts to vitrification. *Theriogenology* 69: 1013-21.
46. Block J, Bonilla L, Hansen PJ (2009) Effect of addition of hyaluronan to embryo culture medium on survival of bovine embryos *in vitro* following vitrification and establishment of pregnancy after transfer to recipients. *Theriogenology* 71: 1063-71.
47. Rasmussen S, Block J, Seidel GE Jr, Brink Z, McSweeney K, et al. (2013) Pregnancy rates of lactating cows after transfer of *in vitro* produced embryos using X-sorted sperm. *Theriogenology* 79: 453-61.
48. Ghys E, Dallemagne M, De Troy D, Sauvegarde C, Errachid A, et al. (2015) Female bovine blastocysts are more prone to apoptosis than male ones. *Theriogenology* 85: 591-600.
49. Liu X, Hu T, Sun W, Hao H, Liu Y, et al. (2015) Comparison of the developmental competence and quality of bovine embryos obtained by *in vitro* fertilization with sex-sorted and unsorted semen from seven bulls. *Livestock Science* 181: 263-70.
50. Stewart BM, Block J, Moelli P, Navarette AE, Amstalden M, et al. (2011) Efficacy of embryo transfer in lactating dairy cows during summer using fresh or vitrified embryos produced *in vitro* with sex-sorted semen. *J Dairy Sci* 94: 3437-45.
51. Jo HT, Bang JI, Kim SS, Choi BH, Jin JI, et al. (2014) Production of female bovine embryos with sex-sorted sperm using intracytoplasmic sperm injection: efficiency and *in vitro* developmental competence. *Theriogenology* 81: 675-82.
52. Li J, Zhu S, He X, Sun R, He Q, et al. (2016) Application of a microfluidic sperm sorter to *in vitro* production of dairy cattle sex-sorted embryos. *Theriogenology* 85: 1211-8.

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