Anim. Reprod., v.3, n.1, p.19-28, Jan./Mar. 2006

Factors influencing *in vitro* embryo production

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Abstract

In vitro embryo production (IVP) is currently one of the most important biotechnologies in cattle breeding and husbandry. However, the efficiency of *in vitro* embryo production is still low with only 30-40% of oocytes developing into blastocysts, probably because, the *in vitro* environment cannot mimic *in vivo* environment and results in embryos that have altered morphology and gene expression. Several factors can influence the IVP efficiency and contribute to the existing differences between *in vivo* and *in vitro* produced embryos. There is also evidence showing that IVP can cause some disorders during gestation and in offspring. The aim of this review is to give a brief overview of some factors that influence *in vitro* embryo development in cattle.

Keywords: bovine, *in vitro* fertilization, embryo development, offspring.

Introduction

In vitro embryo production (IVP) is a reproductive biotechnology that has great potential for speeding up genetic improvement in cattle, but it is also an important research tool for animal embryology. The use of IVP by commercial embryo companies has increased, and currently bovine IVP embryos represent a considerable percentage of the total number of cattle embryos produced in the whole world (Thibier, 2005). For instance, in Brazil more than 40% of the transferred cattle embryos in 2004 consisted of IVP embryos. However, there is ample evidence showing that differences between *in vivo* and *in vitro* produced embryos still exist, which involve morphological and molecular aspects that impair IVP efficiency. These differences are probably induced by several factors such as breed, oocyte quality, follicular environment, fertilization, and embryo culture environment. *In vitro* derived embryos usually have darker coloration, a lack of compactness of the cellular mass, premature formation of the blastocoel, alteration in the ratio of the inner cell mass to trophoblast cells, greater mixoploidy, and alterations in gene expression and cell metabolism (Thompson, 1997; Holm and Callesen, 1998; Lechniak *et al.*, 1998; Khurana and Niemann, 2000; Lonergan *et al.*, 2006). These alterations may be involved in the low rate of embryo cryosurvival and phenotypic disorders observed in fetuses and offspring derived from *in vitro* produced embryos. This review summarizes some of the factors involved in successful *in vitro* embryo production in cattle.

Factors that affect *in vitro* embryo development

Maternal factors

The oocyte is the gamete that contributes not only half of the genetic material but also practically all of the cytoplasm to the zygote, supplying the transcripts and proteins necessary for early embryonic development (Schultz, 2002). This cytoplasmic environment offers the correct conditions so that the embryonic genome can be activated, and the embryo continues its development. Studies have shown that maternal transcripts in early embryos may participate in embryonic genome activation, thus influencing the moment that this activation occurs (Vigneault *et al.*, 2004). This latter study identified maternal transcripts of some transcription factors in oocytes and embryos before genome activation and suggests that maternal transcripts remain stored in the embryo cytoplasm for use in genome activation. Promoting the reprogramming of genetic expression pattern (Schultz, 2002), it is obvious that oocyte quality becomes essential to embryonic development before and after genome activation considering the fact that the appropriate embryonic genome activation is a fundamental key for the subsequent embryo development.

Follicle

Oocyte growth inside the follicle is a slow process that lasts about six months in cattle (Lussier *et al.*, 1987). During this period, the oocyte acquires the competence to undergo meiotic maturation by an interaction between the oocyte and the theca and granulosa cells (Miyano, 2003) and accumulates transcripts and proteins that will guide the maturation, fertilization, and initiate embryo development (Kruip *et al.*, 2000). The oocyte quality is, therefore, related to its follicular environment. Studies have established a relationship between follicle size and oocyte

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Received: December 7, 2005
Accepted: August 1, 2006

competence; the competence increases as the follicle enlarges (Lonergan *et al.*, 1994; Kruip *et al.*, 2000). Some studies have found greater rates of embryonic development using oocytes aspirated from follicles greater than 2-3 mm in diameter (Yang *et al.*, 1998). Hendriksen *et al.* (2000) reported that oocyte competence increased in follicles greater than 8 mm. The low developmental rates of oocytes from small follicles may be because they still do not reach complete meiotic and/or cytoplasmic competence, or because they are from follicles already undergoing atresia. Therefore, follicle health seems to be important in this process since oocytes from follicles in the advanced stage of atresia are more compromised than oocytes from follicles in the early stage of atresia (Hendriksen *et al.*, 2000; Nicholas *et al.*, 2005). Indeed, follicles with the same diameter can be found at diverse stages of the estrous cycle and can be either undergoing growth or atresia. Therefore, the health of follicles may be more important for the competence of oocyte than their diameter.

Oocyte diameter

During the growth phase, the oocyte increases in diameter to more than 120 µm (Hytell *et al.,* 1997). Studies have shown that oocytes with a diameter of less than 110 µm may still be in the growth phase (Fair *et al.*, 1995) and are less able to develop after fertilization. It has been suggested that the critical diameter for an oocyte to acquire developmental competence is 110 µm, which corresponds to oocytes associated with follicles 3 mm in diameter (Hyttel *et al.*, 1997; Fair, 2003). Such small oocytes are also prone to undergo chromosome alterations during maturation, which impairs their further development (Lechniak *et al.*, 2002).

Environment

Heat stress has been shown to be harmful to bovine oocytes and embryos (Al-Katanani *et al.*, 1999; Wolfenson *et al.*, 2000). Holstein cows have lower reproductive performance in autumn than in winter, which is likely a late effect of high temperatures during the summer (Wolfenson *et al.*, 2000). Similarly, oocytes obtained at the beginning of autumn are of low quality, and quality improves gradually as the winter approaches (Roth *et al.*, 2001). It was observed that even cooling cows for 42 days after heat stress was not sufficient to improve *in vitro* embryo production (Al-Katanani *et al.*, 2002), suggesting a late effect of heat on oocyte competence. Moreover, the effect of heat stress may also interfere with follicular development and with the secretion of hormones such as LH and progesterone (Rensis and Scaramuzzi, 2003), thus causing alterations in oocyte quality.

Age

Oocyte developmental competence, which seems to be dependent on age of the donor, is lower in prepubertal heifers than in cows. Prepubertal Holstein heifers between 7-11 months of age have produced oocytes with similar competence to those of cows (Presicce *et al.*, 1997; Majerus *et al.*, 1999) while oocytes from 3-4 month-old calves were less competent than oocytes from their adult counterparts (Khatir *et al.*, 1996; Palma *et al.*, 2001). Oocytes from 4-7 month-old crossbred *Bos indicus heifers* were also less likely to develop into blastocysts after *in vitro* fertilization than oocytes from adult cows, but oocytes from 9-14 month-old crossbred heifers were as competent as the adults' oocytes (Camargo *et al.*, 2005). Differences in energy metabolism and oocyte size (Steeves and Gardner, 1999), as well as in the activity of cytoplasmic factors important for maturation (Salamone *et al.*, 2001) between oocytes from 2-6 month-old calves and adult cows, may account for the low developmental competence of prepubertal oocytes. Hormonal stimulation of donors can be used to increase developmental competence of prepubertal oocytes by enhancing cytoplasmic maturation and increasing the number of follicles available for puncture on the ovarian surface (Armstrong *et al.*, 2001), but the blastocyst production rate may remain inferior to those obtained using oocytes from mature cows (Presicce *et al.,* 1997).

Breed

The effect of breed on developmental competence of oocytes and embryos is evident in some species. In mice, embryos from some strains develop *in vitro* until the blastocyst stage while embryos from others halt development between the 1 and 2-cell stage (Goddard and Pratt, 1983). This difference may be caused by some maternal genetic or cytoplasmic factor (Bavister, 1995). In cattle, the oocyte source, whether from a dairy or a beef breed, influences the blastocyst rate (Fischer *et al.*, 2000; Boediono *et al.*, 2003). The effect of breed on oocyte quality becomes more evident when associated with environmental conditions. Rocha *et al.* (1998) reported that Holstein cows (*Bos taurus*) produced oocytes of lower quality than Brahman cows (*Bos indicus*) during the summer. Similarly, induced heat stress in *in vitro-fertilized* embryos at the 4- and 8-cell stage decreased the blastocyst rate in both Holstein and Brahman breeds; however, the effect was more severe in Holstein embryos (Block *et al.*, 2002; Paula-Lopes *et al.*, 2003).

It is widely known that *Bos indicus* breeds have a great ability to control their body temperature (Hansen, 2004). This feature may be a genetic adaptation at the cellular level that allows *Bos indicus* cattle to better survive in a hotter climate (Paula-Lopes

et al., 2003), resulting in a higher oocyte developmental competence than *Bos taurus cattle* when kept in this environment.

The effect of heterosis on *in vitro* embryo production was evaluated by using gametes from *Bos taurus* and *Bos indicus* donors (Fischer *et al.*, 2000). A greater blastocyst rate for *Bos* taurus purebred than for *Bos* indicus and crossbred cattle was found, yielding a negative heterosis estimate of 45%. This suggests some incompatibility between the *Bos taurus* and *Bos indicus* genome that impairs development of *in vitro* produced crossbred embryos. This could mean that using *in vitro* embryo production would be less efficient in producing *Bos indicus* and F1 embryos. However, *in vitro* embryo production carried out in Brazil has shown that blastocyst development of Gyr embryos (*Bos indicus*) is greater than Holstein embryos (Camargo *et al.*, 2006) and that the development of crossbred embryos (Gyr oocytes with Holstein sperm: 27.3% blastocysts; n = 385 oocytes) may be similar or even higher than that observed for *Bos taurus* purebred embryos (Holstein embryos: 10.8% blastocysts; n = 390 oocytes) when oocytes are obtained by ovum pick-up. Obviously, climate and oocyte donor background must be taken into consideration when performing comparisons, but this shows that *in vitro* embryo production systems are now adapted for *Bos indicus* cattle.

Individual variation

Differences in blastocyst production rate among females have been reported in some studies (Kruip *et al.*, 1994). Tamassia *et al.* (2003) found great variation in the number of oocytes recovered and in the blastocyst rate; nevertheless, an elevated number of oocytes did not necessarily result in a greater blastocyst rate. Therefore, the individual effect of a donor on oocyte developmental competence should be also taken into consideration in an *in vitro* embryo production program.

Paternal factors

Sperm fertility is one of the main factors for fertilization success, and differences in embryo production rate among bulls are commonly reported (Larsson and Rodriguez-Martinez, 2000; Camargo *et al.* 2002a). Spermatozoa need to undergo a sequence of physiologic modifications, termed capacitation, that allow them to penetrate the zona pellucida and fuse with the oocyte cell membrane. Capacitation can be induced *in vitro*; however, while undergoing *in vivo* fertilization, the spermatozoa to oocyte ratio is generally low (around 1:1). With *in vitro* fertilization though, the ratio can reach 20,000:1 (Gordon, 1994). When spermatozoal concentrations are adjusted according to a specific bull's optimal concentration for *in vitro* fertilization, embryo production can be optimized and decrease the differences in blastocyst production rate among bulls (Camargo *et al.* 2002a; Lu and Seidel, 2004).

Another aspect that should be taken into account is the genetic information transmitted by spermatozoa to the embryo. The expression of compromised genetic information from the spermatozoa can impair embryo quality (Leibfried-Rutledge, 1999) and interfere with an *in vitro* embryo program's success.

Factors associated with the in vitro environment

Studies involving *in vitro* culture environments of oocytes and embryos have been carried out by many researchers for more than 30 years, but there still are many questions regarding the effects of *in vitro* culture on embryo development and phenotypic features observed in offspring generated by *in vitro* embryo production. Although the mammalian embryo has great plasticity, which allows it to survive in vitro, it usually shows low quality and viability when under *in vitro* environments (Lane, 2001). Some nutrients have been used in the culture media in an attempt to reach the embryos nutritional requirements, but unfortunately media for embryo development is still not optimized and may cause molecular and phenotypic alterations in embryos, fetuses, and neonates (Farin *et al.*, 2006; Lonergan *et al.*, 2006).

Culture systems

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 replaced by albumin; or fully defined, a protein-free system where albumin is replaced by macromolecules such as polyvinyl alcohol and polyvinyl pyrrolidone (Marquant-Le Guinne and Humblot, 1998; Farin *et al.*, 2001; Vanroose *et al.*, 2001). 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. Embryos can also be cultured in microchannels using a microfluidic device that allows embryo culture within a smaller volume than usual and gradual replacement of the culture medium without embryo manipulation. This approach seems to provide a better *in vitro* environment (Beebe *et al.*, 2002; Quinn, 2004).

Undefined culture system

In the undefined culture system, serum is one of the main components. It can provide many beneficial factors to the embryo such as amino acids, vitamins, growth factors, and energetic substrates; however, it may also contaminate the culture media with embryotoxic factors (Bavister, 1995). Serum usually

increases the blastocyst rate (Lim *et al.*, 1994; Gomez and Diez, 2000) by a biphasic effect, inhibiting the first cell divisions and stimulating further embryo development (Bavister, 1995; Camargo *et al.*, 2002b). Despite increasing blastocyst yield, serum also increases the accumulation of cytoplasmic lipids, reduces embryo survival after cryopreservation (Abe *et al.*, 2002; Rizos *et al.*, 2003), increases the male to female embryo ratio (Gutiérrez-Adan *et al.*, 2001), and disturbs gene expression (Wrenzycki *et al.*, 2001; Rizos *et al.*, 2003). Its use in culture media has been implicated in diverse phenotypic alterations observed during gestation and in bovine newborns such as placental defects and large offspring (Young *et al.*, 1998; McEvoy, 2003). In mice, it was shown that *in vitro* culture of embryos with serum also alters the gene expression, fetal development, and post-natal behavior (Fernandez-Gonzalez *et al.*, 2004).

Co-culture with somatic cells can also be used in undefined culture systems. Some commercial labs have used co-culture to produce bovine embryos, especially in Brazil, where more than 50,000 embryos are produced annually. Somatic cells may contribute to embryo development by removing harmful substances such as heavy metals as well as secreting embryotrophic factors such as growth factors (Bavister, 1995). Nevertheless, co-culture may also have some disadvantages; somatic cells can be the source of variation in embryo production among batches when different kinds of cells as well as cells from different animals are used. Moreover, somatic cells usually used in co-culture are susceptible to viral contamination such as bovine viral diarrhea (Waldrop *et al.,* 2004) and bovine herpes virus-1 (Vanroose *et al.,* 1999), and such contamination may influence *in vitro* culture environments by affecting embryo development, even without viral replication in embryonic cells (Vanroose *et al.,* 1999).

Semi-defined culture systems

Due to suspicions of detrimental effects of serum on embryonic and fetal development (Young *et al.*, 1998), serum-free culture systems have been investigated. A semi-defined culture system is generally performed by replacing serum with albumin, thus eliminating many potentially harmful components of serum (Bavister, 1995). Albumin is one of the most prevalent proteins of the mammalian reproductive tract and it may have a nutritive role during embryo developmental post-compaction (Thompson, 2000). Studies have shown that bovine embryos can be cultured in serum-free medium with very low amounts of BSA (Krisher *et al.*, 1999). Another study found that BSA in culture medium produced embryo with greater viability after vitrification compared to serum (Rizos *et al.*, 2003). Embryos with great viability after cryopreservation were also obtained when oocytes were matured and embryos cultured in a culture medium with 1 mg/ml BSA (Abe *et al.*, 2002), causing less deviation in newborn weight (Hoshi, 2003). Nevertheless, BSA is still a biologic component subject to contamination that may impair embryo and fetal development, and its role in *in vitro* culture is not very clear (Bavister, 1995; Thompson, 2000). A possible role of BSA in embryonic development may be to provide amino acid substrates for embryo metabolism, which could favor embryo development (Orsi and Leese, 2004). Recently, Miles *et al.* (2005) reported alteration in the early development of placentae when bovine embryos were cultured in a semi-defined system; however, gestational defects may be associated with BSA from different companies (Peterson and Lee, 2003). An alternative for reducing BSA contamination is to use recombinant BSA, which may provide similar blastocyst and survival rates after cryopreservation (Lane *et al.*, 2003b).

Defined culture systems

It has been shown that *in vitro* fertilized bovine embryos can develop in protein-free culture systems (Pinyopummintr and Bavister, 1991; Keskintepe *et al.*, 1995; Holm *et al.*, 1999). The advantage of this system is that it eliminates the potential harmful effects of serum, co-culture, and albumin on *in vitro* fertilized embryos. It also allows better control of culture conditions, facilitating studies designed to evaluate embryo culture requirements. However, blastocyst yield using defined culture systems has not been consistent among studies and has frequently lowered embryo production compared to semi-defined systems (Lonergan *et al.*, 1999; Kuran *et al.*, 2001; Orsi and Leese, 2004), which has limited the commercial use of this kind of culture system. Because it does not have the protective action of serum or co-culture, defined systems may become more sensitive to toxic contamination and oxidative stress.

Glucose

Glucose is the main energetic substrate consumed by cells; however, it elicites harmful effects on the early development of pre-implantation mammalian embryos (Bavister, 1995). Pyruvate and lactate are the preferential energetic substrates consumed by the early embryo during *in vitro* development (Pinyopummintr and Bavister, 1996) with higher lactate oxidation until the 8-cell stage than *in vivo* embryos (Khurana and Niemann, 2000). Glucose uptake only increases after compaction and is metabolized mainly to lactate (Sinclair *et al.*, 2003). At early cleavage stages, glucose might impair embryonic development, inhibiting oxidative phosphorylation through glycolytic metabolites (Bavister, 1995) when embryos depend mainly on this route for generating energy (Thompson, 2000). Increased glucose

concentration may also cause deviation in embryo male to female ratio (Bredbacka and Bredbacka, 1996) by retarding the development of female embryos. This effect may be caused by glucose-induced differential expression of X-linked genes between the genders resulting in different developmental conditions for male and female embryos (Bredbacka and Bredbacka, 1996). Over-expression of the gene that encodes glucose 6-phosphate dehydrogenase, an X-linked gene, may be caused by excessive glucose, increasing the pentose-phosphate pathway activity in female embryos (Kimura *et al.*, 2005) and retarding their development. Nevertheless, glucose suppression from a culture medium does not seem like the best way to avoid such an effect because this substrate is important to ribose synthesis as well as to NADPH production (Bavister, 1995; Thompson, 2000); therefore, low glucose concentration has been used in embryo culture media instead of glucose suppression.

Oxygen tension

The oxygen tension that most mammalian embryos encounter in the reproductive tract range from 3.5 to 8% (Fischer and Bavister, 1993). It has been shown that embryos can also be cultured *in vitro* using a similar oxygen tension in a cell-free culture system (Thompson *et al.*, 1990; Watson *et al.*, 1994).

The positive effect of low oxygen tension on embryo culture has been reported for many species. For instance, the culture of *in vitro* fertilized pig embryos in low oxygen increased the number of cells (Booth *et al.*, 2005) and embryo production rate (Karja *et al.*, 2004) to greater than those cultured in 20% oxygen. In mice, culture in 20% oxygen resulted in disruptions in fetal development (Karagenc *et al.*, 2004), but not in 5% oxygen. The reduction of oxygen tension to 5% has also been shown to improve development of *in vitro* fertilized bovine embryos (Thompson *et al.*, 1990) resulting in less embryos halted at the 8-16 cell stage (Liu and Foote, 1995). High oxygen tension may affect the maternal zygotic transition, increasing the length of the fourth cell cycle in most embryos (Lequarre *et al.*, 2003). Low oxygen tension in embryo culture may contribute to reducing free-radical formation. Free radicals impair embryo metabolism and development (Lane, 2001). High oxygen tension may cause apoptosis in embryonic cells (Yuan *et al.*, 2003) and/or alter the embryonic gene expression pattern (Harvey *et al.*, 2004). Nevertheless, culture in 5% oxygen may decrease the proportion of ICM:TE cells (Fischer-Brown *et al.*, 2002) although this effect on ICM cells may vary between species (Karagenc *et al.*, 2004).

The effects of oxygen tension on the development of *in vitro* fertilized embryos seem to be dependent on the culture media or culture systems used. Lower blastocyst yield may be found in undefined systems with co-culture in TCM199 and using 5% rather than 20% oxygen (Voelkel and Hu, 1992). Nevertheless, in a cell-free SOF medium with serum, embryo development and survival after cryopreservation were favorable using 5% oxygen when compared to 20% oxygen (Rizos *et al.*, 2001), but no difference was observed when culture was performed in TCM199 (Khurana and Niemann, 2000).

Defined and semi-defined culture systems generally require low oxygen tension (5%) to yield higher blastocyst rates (Vanroose *et al.*, 2001). Lonergan *et al.* (1999) observed that culturing bovine embryos in SOF and in SOF plus BSA using 5% oxygen compared to 20% increased the blastocyst yield on Day 8. Similarly, Lane *et al.* (2003a) reported a higher blastocyst rate using 5% oxygen and protein-free G1.1/G1.2 sequential medium. High oxygen tension may be more harmful to embryos in a defined and semi-defined culture system compared to an undefined system because of increased production of free oxygen radicals, likely due to increased oxidative stress (Bavister, 1995; Vanroose *et al.*, 2001).

Oxygen tension may also influence post-transfer development of *in vitro* fertilized embryos. Iwata *et al.* (2000) reported that birth weight had been heavier when 5-cell stage bovine embryos were cultured in 20% oxygen (air) rather than 5% oxygen. Fischer-Brown *et al.* (2005) reported that embryos cultured in a semi-defined system in 20% oxygen had cotyledons with an increased area and size. Calves also tended to be heavier than those from embryos cultured in 5% oxygen. The authors observed that this last effect was evident when embryos were cultured in KSOM when compared to SOF.

Amino acids and growth factors

Secretions of the female reproductive tract have several amino acids that can be used as energetic substrate by the embryo (Bavister, 1995). The use of amino acids in serum-free culture media improves embryo development (Pinyopummintr and Bavister, 1996; Lee *et al.*, 2004), probably through an antioxidant action (Liu and Foote, 1995) and controlling pH and osmolarity (Gardner, 1998). Amino acids can also reduce the stress and cell fragmentation caused by *in vitro* embryo culture (Donnay *et al.*, 1999). Nevertheless, amino acid metabolism releases ammonium into the culture medium, which is harmful to embryos. Because of this side effect, the culture medium needs to be replaced by a new one after two or three days of embryo culture (Thompson, 2000).

There is some evidence that growth factors secreted by cells stimulate mammalian embryo development (Ghosh and Sengupta, 1998). Currently, several studies suggest that growth factors in cell-free culture medium improve blastocyst rate (Byrne *et al.*, 2002; Sinclair *et al.*, 2003). Epidermal growth factor (EGF) improves nuclear maturation and cleavage rate

(Rieger *et al.*, 1998) as well as embryo development (Sirisathien *et al.*, 2003). Insulin-like growth factor 1 (IGF-1) and 2 (IGF-2) increases blastocyst rate and cell number (Byrne *et al.*, 2002; Sirisathien *et al.*, 2003). Furthermore, growth factors can stimulate mitosis (IGF-1), reduce apoptosis (IGF-1 and –2), and stimulate protein synthesis (EGF; Diaz-Cueto and Gerton, 2001; Byrne *et al.*, 2002; Sirisathien *et al.*, 2003), functioning through an autocrine or paracrine mechanism (Diaz-Cueto and Gerdon, 2001; Hardy and Spanos, 2002). Other growth factors may also have beneficial effects on embryo development (Ghosh and Sengupta, 1998; Diaz-Cueto and Gerton, 2001; Hardy and Spanos, 2002).

Sequential medium

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 tract during pre-implantation development seems promising. In human embryos, sequential medium improves development until the blastocyst stage, offering the option to transfer only one embryo to a mother's womb instead of three or more embryos at earlier stages, thus avoiding multiple gestations (Gardner and Lane, 2003). Currently, there are different sequential media available for culturing human embryos such as G1.2/G2.2 (Gardner, 1994) and M1/M2 (Zollner *et al.*, 2004). In cattle, sequential media seems promising for replacing media with serum and/or co-culture. Lane *et al.* (2003a) observed similar results in embryo production and gestation between protein-free G1.2/G2.2 sequential medium and BRL co-culture with 10% serum; however, the male to female ratio was not altered in G1.2/G2.2 medium whereas the male percentage was higher in co-cultured embryos. Culturing bovine embryos in KSOM medium until Day 3 post-fertilization with the addition of 1mg/ml BSA, followed by SOF with 10 mg/ml BSA increased the blastocyst rate and the cell number (Nedambale *et al.*, 2006).

In conclusion, *in vitro* production of bovine embryos is an efficient tool to produce animals of higher genetic merit, and its use has increased, mainly in developing countries in South America and Asia. Several factors can influence the success of *in vitro* embryo production and increase or decrease the embryo yield and gestational viability. Differences between donors, sperm, and breeds should be taken into account in regard to *in vitro* embryo production. Using breeds adapted to the environment will certainly overcome the effects of heat on oocyte quality and embryo yield. *In vitro* culture environment is another factor that greatly influences embryo production. There are different culture systems and culture media available for *in vitro* fertilized bovine embryos, and embryo yield generally varies among them. The efficiency of culture systems may also vary among labs, making data comparisons difficult. Therefore, it may be advisable to test what culture system or culture medium will provide better results for a given lab.

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