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Effect of Two Different Culture Media on Developmental Rate of Bovine Embryos *invitro*

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A B S T R A C T

In the present study, effect of two different culture media on the developmental competence of bovine oocytes was studied. For the study a total of 1110 oocytes were collected using ovum pick-up (OPU) technique and the average oocyte yield was 8.34 ± 0.23 per OPU session per animal. The oocytes were graded as A, B, C and D grades based on the layers of compact cumulus cells surrounding the zona pellucida. Among this only A, B and C grade oocytes were used for *in vitro* maturation. The oocytes were matured in TCM 199 media supplemented with 10 per cent FBS, 1 µg/ml Folltropin (Bioniche, Canada), 0.02 IU/ml LH, 1 µg/ml estradiol with addition of 100 µM cysteamine, 10 ng/ml EGF, 100 ng/ml IGF, 10 µg/ml insulin, 5.5 µg/ml transferrin and 5 µg/ml selenium and the overall maturation rate was 91.30± 1.27 per cent. The presumptive zygotes were cultured in two different culture media viz. i) two step sequential synthetic oviduct fluid (SOF) media and ii). single step potassium simplex optimised medium (KSOM). The mean (± SE) cleavage, 4 cell, 8 cell and morula development rate was 73.04 ± 1.50 and 79.01 ± 1.34 per cent, 54.88 ± 1.76 and 60.69 ± 1.89 per cent, 35.89 ± 1.57 and 44.67 ± 1.62 per cent and 19.18 ± 1.10 and 25.36 ± 1.37 per cent in SOF media and KSOM, respectively. Based on the cleavage and embryo development observed, it was concluded that KSOM could be better than SOF media for *in vitro* production of bovine embryos.

Introduction

Assisted Reproductive Technology (ART) is a biotechnological tool which has been employed to improve the reproductive efficiency like infertility in human beings and production of high genetic merit animals. *In vitro* fertilization is one of the assisted reproductive technologies used for the multiplication of genetically superior animals and the preservation of genetics (Lonergan, 2007).

The successful application of ART and

related technologies are critically dependent on basic techniques like *in vitro* maturation of oocytes, *in vitro* fertilization and *in vitro* culture of embryos. Without significant improvements of these ART techniques, application of developments in cloning and the production of transgenic farm animals will remain limited and extremely costly (Galli *et al.,* 2014).

After introduction of ultrasonic guided aspiration of bovine follicular oocyte (Callesen *et al.,* 1987 and Pieterse *et al.,* 1991), a significant improvement was observed in terms of recovered oocytes and *in vitro* embryo production. Oocyte quality determines the developmental competence of an embryo and it depends on the oocytes nuclear and cytoplasmic maturation (Sirard *et al.,* 2006).

In vitro culture systems play a vital role in supporting *in vitro* development of pre implantation embryos (Thompson *et al.,* 2007). The use of defined culture media is necessary to acquire a better comprehension of metabolism and biochemical requirements for *in vitro* embryo production (IVEP). It is well established that embryo metabolism, cleavage, and pregnancy rates are affected by the media composition, which may lead to a diminished embryonic and fetal developmental capacity (Camargo *et al.,* 2006).

With the above background, the present study was conducted to investigate the effect of two different *in vitro* culture systems on the developmental competence and quality of bovine embryos.

Materials and Methods

All reagents used for the preparation of media were from Sigma (Sigma–Aldrich), unless stated otherwise. Freshly made media were stored at 4°C and were used up to 1 month.

Oocyte collection by ovum pick-up

For the study, bovine oocytes were collected from live animals using ovum pick-up (OPU) technique using a real-time B mode ultrasound scanner (SA-600V; Kretztecknik AG, BCF Technology, Scotland) equipped with a 6.5 MHz curved array (convex) transvaginal probe (VE5-8/20R, 6.5 MHz/20R/86D, BCF Technologies, Scotland). It was used for scanning of ovaries and subsequent follicular aspiration.

Twelve physiologically normal crossbred cows were used as oocyte donors in this experiment. The cows were regularly cyclic and in optimal nutritional status. The oocyte collection was carried out on every Monday, Wednesday and Friday. On a particular day/session, four animals were used for collection. Before the start of OPU, the animals were restrained in the trevis and the perineal area was cleaned with running tap water and with 70% alcohol. The epidural anesthesia was given with 5 ml of 2% lignocaine hydrochloride. The ovaries were manipulated per rectum and either the right or left ovary was positioned between the fingers and the needle was inserted in the guide and advanced through the fornix vagina and into the follicle antrum. The follicles of >2 mm dia. were aspirated through the transvaginal probe using pre-equilibrated (38.5°C) oocyte collection medium (modified HEPES – buffered tyrodes medium, pH 7.2-7.4) supplemented with 0.3 per cent BSA, 20 IU/ml heparin, (Beparine®, Biological E Ltd.,) and 1 per cent penicillin - streptomycin. After aspiration, the collection tube with contents of aspirated follicles was transported to the laboratory within 5 min. of collection and kept undisturbed for 5 min. The follicular fluid was observed under a zoom stereo microscope (Nikon, Japan) for the presence of cumulus oocyte complexes (COCs). The COCs were transferred into another petridish containing oocyte collection medium. The

collected immature COCs were categorized into A, B, C, and D grades as described by Cetica *et al.,* (1999). Among the four grades only grade A (COCs with more than 5 layers of compact cumulus cells surrounding the zona pellucida), grade B (COCs with 3-5 layers of cumulus cells surrounding the zona pellucida) and grade C (COCs with $\leq$ 3 layers of cumulus cells surrounding the zona pellucida) oocytes were subjected to *in vitro* maturation. Grade D oocytes (completely devoid of cumulus mass and having irregular and dark ooplasm) were discarded.

Experimental design

Study 1

Studied the effect of different grades of COCs on *in vitro* maturation of bovine oocytes.

Study 2

Evaluated the effects of two different culture media on *in vitro* developmental competence of bovine embryos.

In vitro culture of presumptive zygotes in two step sequential synthetic oviduct fluid (SOF) media

In vitro culture of presumptive zygotes in single step potassium simplex optimised medium (KSOM)

In vitro maturation

The *in vitro* maturation of the selected oocytes was carried out in TCM 199 medium supplemented with 10 per cent FBS, 1 µg/ml Folltropin (Bioniche, Canada), 0.02 IU/ml LH, 1 µg/ml estradiol with addition of 100 µM cysteamine, 10 ng/ml EGF, 100 ng/ml IGF, 10 µg/ml insulin, 5.5 µg/ml transferrin and 5 µg/ml selenium. Grade A, B and C immature oocytes were transferred into each droplet (10 COCs per droplet) and incubated in a CO_2 incubator maintained in 5 per cent CO_2, 38.5^{o}C with 90-95 per cent relative humidity for 24h. After 24h of incubation in the maturation media, the degrees of cumulus expansion of the oocytes were ascertained under zoom stereo microscope and the *in vitro* maturation was assessed based on cumulus cell expansion (Figure 1). Oocytes with full cumulus cell expansion (degree 2) and moderate cumulus expansion (degree 1) were considered as matured and oocytes with slight or no expansion of cumulus cell mass (degree 0) were considered as not matured (Kobayashi *et al.*, 1994).

In vitro fertilization

Frozen semen from single bull was used for the entire study. For each trial, four frozen semen straws (0.25 ml French mini) were thawed in a water bath containing water at 37°C for 30 seconds. The straws were removed from water bath, wiped with 70 per cent alcohol and the contents were collected in a sterile glass test tube. The progressively motile sperms for *in vitro* fertilization were separated by swim up technique as described by Parrish *et al.,* (1986). In brief, the contents of semen straws were diluted with 5 ml of pre-equilibrated sperm TALP (SpTALP) (Totey *et al.,* 1993) by centrifugation at 350 g for 5 min. at room temperature. The supernatant was removed and fresh SpTALP was added and the above procedure was repeated twice. Finally 100 µl of the sperm pellet was layered under 1 ml of SpTALP medium in three sugar tubes and incubated for swim up in CO_2 incubator maintained at 5 per cent CO_2, 38.5^{o}C with 90-95 per cent relative humidity for one hour. At the end of incubation the superficial layer of 0.5 ml of the medium containing the motile fraction was removed from each tube and pooled in a 15 ml centrifuge tube, and washed with 5 ml SpTALP by centrifugation at 350 g for 5 min

at room temperature. Concentration of the final sperm pellet was determined with a haemocytometer and the sample was diluted with SpTALP to yield a concentration of 1-2 $\times 10^6$ sperms/ml.

After 24h of maturation the degree 2 and degree 1 oocytes were washed thrice in oocyte collection media to remove any extraneous surrounding cumulus layers, followed by final washing in IVF TALP medium. Then about 8-10 matured oocytes were placed in each IVF droplets and incubated to 18-20h in CO_2 incubator.

In vitro culture

The cumulus cells from the fertilized oocytes were removed by manual repeated pipetting. The presumptive zygotes were washed three times in *in vitro* culture medium to remove the spermatozoa, cellular debris and chemical residues and transferred randomly into pre-equilibrated 50µl IVC droplets (10-15 presumptive zygotes/droplet). In each trial one batch of presumptive zygotes were cultured in two step SOF media and another batch in single step KSOM at 38.5°C in 5 per cent CO_2 in air. In two step SOF media, the presumptive zygotes were cultured in early-stage medium (SOF supplemented with 0.5mM glucose) for the 72h and then transferred to later-stage media (SOF without glucose). In the single step KSOM, the presumptive zygotes were cultured in pre-equilibrated KSOM. The cleavage rate and developmental competence of early embryos to morula/blastocyst (Figure 2 and 3) were assessed once in 24 hours. The collected data were analyzed statistically.

Results and Discussion

In the present study, a total of 1110 oocytes were retrieved by OPU. The average oocyte yield was 8.34 ± 0.23 per OPU session per animal. The average yield (Mean ± SE) of A, B and C grade oocytes were 3.49 ± 0.11, 2.51 ± 0.17 and 1.21 ± 0.06 per OPU session per animal, respectively giving a total of 7.21 ± 0.20 usable oocytes per OPU session per animal. The yield of D grade oocytes per OPU session per animal was 0.53 ± 0.45.

In accordance to this study, Looney *et al.,* (1994) and Karadjole *et al.,* (2010) collected an average of 6.3 and 6.5 oocytes, respectively per aspiration in cows. When compared to this study, Garcia and Salaheddine (1998) and Manik *et al.,* (2003) obtained a lower recovery rate of 5.6±1.18 and 4.0±0.5 oocytes in Holstein Friesian and Karan Fries cows, respectively. Merton *et al.,* (2003) reported that oocyte recovery rate through trans-vaginal follicular aspiration has been variable among laboratories and experience of the operator and his team. Oliveira *et al.,* (2016) reported that genetics of the donor, vacuum pressure while performing aspiration, type of needle used for OPU, and number of follicles present on the ovary was some of the factors affecting the recovery rate of oocytes. These factors may influence the quality of oocytes collected, success of *in vitro* maturation, *in vitro* fertilization and subsequent *in vitro* embryo development.

In order to obtain more oocytes from the donor, it was necessary to have more follicles on the cow ovary (Ward *et al.,* 2000). Gimenes *et al.,* (2015) reported that the number of follicles on the ovary was influenced by breed of cattle, nutritional status of cow and climatic conditions. Wolfenson *et al.,* (2000) reported that the temperature played a key role on bovine follicle formation and development, oocyte quality and embryo development and they also stated that heat stress suppressed follicular dominance, resulting in a number of changes in follicular growth. The number

oocytes matured and maturation rate obtained in the present study is presented in table 1. *In vitro* maturation of different grades of COCs was carried out in TCM 199 media supplemented with 10 per cent FBS, 1 µg/ml Folltropin (Bioniche, Canada), 0.02 IU/ml LH, 1 µg/ml estradiol with addition of 100 µM cysteamine, 10 ng/ml EGF, 100 ng/ml IGF, 10 µg/ml insulin, 5.5 µg/ml transferrin and 5 µg/ml selenium. TCM 199 is a standard medium for *in vitro* maturation of bovine oocytes either with serum supplementation (Do *et al.,* 2016) or serum replacer (Moore *et al.,* 2007). Only A, B, C grade bovine oocytes were utilized for *in vitro* maturation and maturation rate was assessed based on the cumulus expansion. Out of 1034 oocytes cultured, 523 showed degree 2 cumulus expansion with a mean maturation rate of 50.57 ± 1.04 per cent, 421 showed degree 1 cumulus expansion with a mean maturation rate of 40.74 ± 1.17 per cent and 90 showed degree 0 cumulus expansion with a mean maturation rate of 8.70 ± 0.98 per cent.

The overall maturation rate obtained in the present study was 91.30± 1.27 per cent (944 oocytes matured out of 1034 oocytes used). In accordance to this study, Lonergon *et al.,* (1996) reported 91 per cent of maturation rate in bovine oocytes matured with EGF (10 ng/ml) supplemented media. The overall oocyte maturation rate observed in the present study in TCM199 media was higher than the maturation rates reported by other researchers viz., 70.20 per cent (Lonergon *et al.,* 1994) and 75.53 per cent (Pontes *et al.,* 2011) from OPU derived COCs.

The higher maturation rate obtained in this study might be due to supplementation of *in vitro* maturation medium with EGF and IGF which showed positive influence on cumulus expansion (Kelly *et al.,* 2008). The developmental rate of bovine presumptive zygotes in SOF media and KSOM is presented in table 2. Out of 471 presumptive zygotes cultured under SOF media, 344 cleaved into 2 cell stage with a cleavage rate of 73.04 ± 1.50 per cent. In KSOM, 473 presumptive zygotes were cultured and 374 were cleaved with a cleavage rate of 79.01 ± 1.34 per cent. Statistical analysis revealed a significantly higher ($P<0.05$) cleavage rate in KSOM when compared to SOF media. In contrast to this study, Zicarelli *et al.,* (2003) reported that there was no significant difference between SOF and KSOM with regard to cleavage rate in buffaloes.

Kim *et al.,* (2014) reported a lower cleavage rate of 65 and 41 per cent in KSOM and SOF media, respectively when bovine embryos cultured *in vitro*. When compared to this study, the cleavage rate of 81 and 82 per cent was reported by Reis *et al.,* (2002) and Rizos *et al.,* (2002) in SOF media and 89 per cent by Felmer *et al.,* (2011) in KSOM. The percentage of embryos (mean ± SE) that progressed to 4 cell stage was significantly ($P<0.05$) higher in KSOM (60.69 ± 1.89) when compared to SOF (54.88 ± 1.76). The mean percentage of embryos that developed to 8 cell stage was 35.89 ± 1.57 and 44.67 ± 1.62 in embryos cultured under SOF media and KSOM, respectively. The progression to 8 cell stage was significantly ($P<0.05$) higher in KSOM than in SOF media.

The percentage of embryos (mean ± SE) that progressed to morula stage was 19.18 ± 1.10 and 25.36 ± 1.37 per cent in embryos cultured under SOF media and KSOM, respectively. The progression to morula stage was significantly ($P<0.05$) higher in embryos cultured in KSOM than in SOF media. Kim *et al.,* (2014) reported 41 and 30 per cent morula development in KSOM and SOF media, respectively when bovine embryos cultured *in vitro* which was higher than the results obtained in this study. Nedambale *et al.,* (2004) obtained morula percentage of 38 in KSOM and 40 in SOF media.

Table.1 No. of oocytes matured and maturation rate (per cent) of bovine oocytes cultured in TCM 199 medium

No. of oocytes used for maturation	No. of oocytes matured (per cent)			Total No. of oocytes matured (per cent)
	Full cumulus cell expansion (Degree 2)	Moderate cumulus cell expansion (Degree 1)	No expansion (Degree 0)	
1034	523 (50.57±1.04)	421 (40.74±1.17)	90 (8.70±0.98)	944 (91.30±1.27)

Table.2 Developmental rate of bovine oocytes in SOF media and KSOM

Culture media	No. of presumptive zygotes cultured	Percentage of presumptive zygotes developed to			
		2 cell	4 cell	8 cell	Morula
SOF	471	73.04±1.50[a]	54.88± 1.76[a]	35.89±1.57[a]	19.18±1.10[a]
KSOM	473	79.01±1.34[b]	60.69±1.89[b]	44.67±1.62[b]	25.36±1.37[b]

Values bearing different superscripts (a, b) within the same column differ significantly ($p < 0.05$)

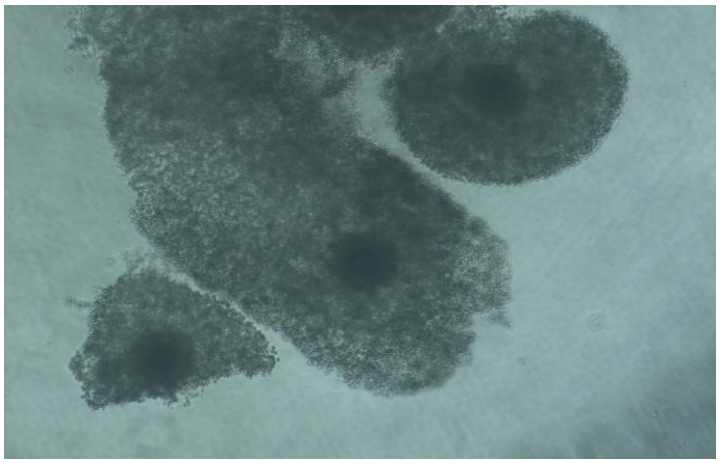

Figure.1 Matured oocytes showing cumulus cell expansion

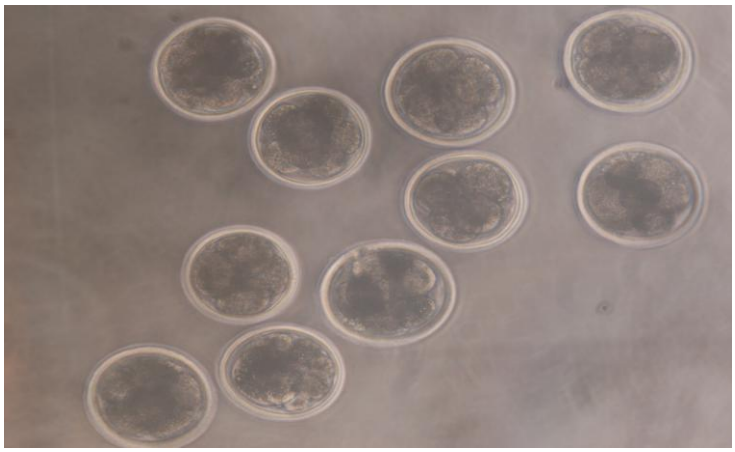

Figure.2 cell embryo

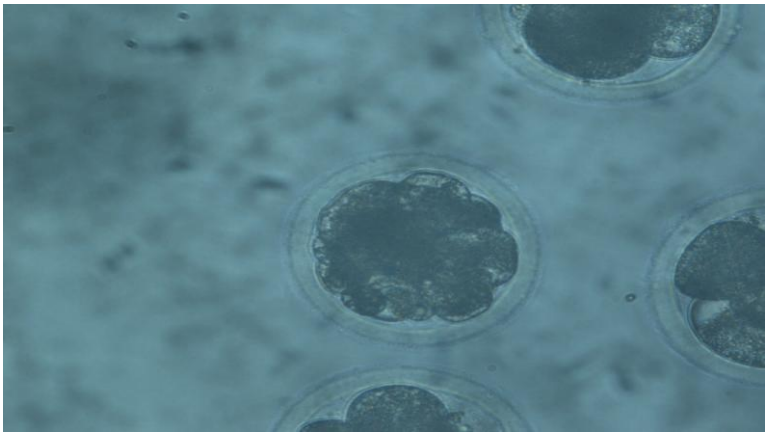

Figure.3 Morula stage embryo

Tervit *et al.,* (1972) had first described the successful *in vitro* culture eight-cell cattle embryos to morula or blastocyst stages in SOF media. This medium was based on the biochemical composition of sheep oviductal fluid. Mouse embryos cultured in KSOM were closer to *in vivo* embryos in terms of gene expression profiling (Doherty *et al.,* 2000). Felmer *et al.,* (2011) observe higher blastocyst formation rate (32 per cent) in KSOM supplemented with BSA. The composition of SOF and KSOM differs slightly. The KSOM had glutamine whereas SOF did not have it. Glutamine has been reported to have an important role in embryo metabolism and blastocyst formation. Summers (2013) reported that glutamine gives an advantage to KSOM.

Since KSOM contained EDTA and a lower concentration of sodium chloride (95 mM) compared to mSOF (EDTA-free and 105 mM sodium chloride), KSOM as basal medium was hypothesized to support better pre-implantation development of bovine embryos than SOF media (Bhuiyan *et al.,* 2004).

The higher cleavage rate and embryo development obtained in the present study also indicated the superiority of the KSOM than the SOF media in the in vitro bovine embryo production as described by Bhuiyan *et al.,* (2004) and Summers (2013).

Ovum pick-up technology can be used effectively to obtain cattle oocytes in large scale.

In vitro embryo production is an efficient procedure for producing embryos from post-puberty heifers and from adult cattle in the laboratory through ovum pick-up and subsequent maturation, fertilization and culture *in vitro*.

The developmental competence of presumptive zygotes is affected by media composition.

The cleavage rate and morula development was higher in KSOM than SOF media.

The KSOM is better due to its composition in terms of presence of EDTA, glutamine and lactic acid in triple concentration when compared to SOF media.

References

Bhuiyan, M.V., J.K. Cho, G. Jang, E.S. Park, S.K. Kang, B.C. Lee and Kwang, W.S. 2004. Effect of protein supplementation in potassium simplex optimization medium on pre-implantation development of bovine non-transgenic and transgenic cloned embryos. Theriogenology. 62: 1403-1416.

Callesen, H., T. Greve and Christensen, F. 1987. Ultrasonically guided aspiration of bovine follicular oocytes. Theriogenology. 27: 217.

Camargo, L.S.A., J.H.M. Viana, W.F. Sa, A.M. Ferreira, A.A. Ramos and Vale Filho, V.R. 2006. Factors influencing *in vitro* embryo production. Animal Reproduction. 3(1):19-28.

Cetica, P.D., L.N. Pintos, G.C. Dalvit and Beconi, M.T. 1999. Effect of lactate dehydrogenase activity and isoenzyme localization in bovine oocytes and utilization of oxidative substrates on *in vitro* maturation. Theriogenology. 51(3): 541-550.

Do, V.H., S. Walton and Taylor-Robinson, A.W. 2016. Improvements to *in vitro* Culture Media for Use in Bovine IVF. Journal of Veterinary Science and Animal Husbandry. 4(2): 1-6.

Doherty, A.S., M.R. Mann, K.D. Tremblay, M.S. Bartolomei and Schultz, R.M. 2000. Differential effects of culture on imprinted H19 expression in the preimplantation mouse embryo. Biology of Reproduction. 62:1526-1535.

Felmer, R.N., M.E. Arias, G.A. Munoz, G.A. and Rio, J.H. 2011. Effect of different sequential and two-step culture systems on the development, quality, and RNA expression profile of bovine blastocysts produced *in vitro*. Molecular Reproduction and Development. 78(6): 403-414.

Fischer-Brown, A.E., B.R. Lindsey, F.A. Ireland, D.L. Northey, R. Monson, S.G. Clark, M.B. Wheeler, D.J. Kesler, S.J. Lane, K.A. Weigel and Rutledge, J.J. 2004. Embryonic disc development and subsequent viability of cattle embryos following culture in two media under two oxygen concentrations. Reproduction Fertility and Development. 16(8): 787-793.

Galli, C., R. Duchi, S. Colleoni, I. Lagutina and Lazzari, G. 2014. Ovum pick-up, intra cytoplasmic sperm injection and somatic cell nuclear transfer in cattle, buffalo and horses: from the research laboratory to clinical practice. Theriogenology. 81(1): 138-151.

Garcia, A. and Salaheddine, M. 1998. Effects of repeated ultrasound guided transvaginal follicular aspiration on bovine oocyte recovery and subsequent follicular development. Theriogenology. 50: 575-585.

Gimenes, L.U., M.L. Ferraz, P. Fantinato-Neto, M.R. Chiaratti, L.G. Mesquita, M.F. Sa Filho, F.V. Meirelles, L.A. Trinca, F.P. Renno, Y.F. Watanabe and Baruselli, P.S. 2015. The interval between the emergence of pharmacologically synchronized ovarian follicular waves and ovum pickup does not significantly affect *in vitro* embryo production in *Bos indicus*, *Bos taurus*, and *Bubalus bubalis*. Theriogenology. 83: 385-393.

Karadjole, M., I. Getz, M. Samardzija, N. Macesic, M. Matkovic, Z. Makek, T. Karadjole, G. Bacic, T. Dobranic and Poletto, M. 2010. The developmental competence of bovine immature oocytes and quality of embryos derived from slaughterhouse ovaries or live donors by ovum pick-up. Veterinarski arhiv. 80(4): 445-454.

Kelly, J.M., D.O. Kleemann, W.M.C. Maxwell and Walker, S.K. 2008. Effects of insulin-like growth factor-I, epidermal growth factor and cysteamine on the *in vitro* maturation and development of oocytes collected from 6- to 8-week-old Merino lambs. Reproduction Fertility and Development. 20(5): 570-578.

Kim, S.W., Y. G. Jung, J. Park and Roh, S. 2014. Comparison of two different serum-free media for *in vitro* culture of bovine embryos. Journal of Animal Reproduction and Biotechnology. 29(3): 229-234.

Kobayashi, K., S. Yamashita and Hoshi, H. 1994. Influence of epidermal growth

factor and transforming growth factor on *in vitro* maturation of cumulus cell enclosed bovine oocytes in a defined medium. Journal of Reproduction and Fertility. 100: 439-446.

Lonergan, P. 2007. State of the art embryo technologies in cattle. Society of Reproduction and Fertility supplement. 64: 315-325.

Lonergan, P., C. Carolan, A. V. Langendonckt, I. Donnay, H. Khatir and Mermillod, P. 1996. Role of epidermal growth factor in bovine oocyte maturation and pre-implantation embryo development *in vitro*. Biology of Reproduction. 54(6): 1420-1429.

Lonergan, P., P. Monaghan, D. Rizos, M. P. Boland and Gordon, I. 1994. Effect of follicle size on bovine oocyte quality and developmental competence following maturation, fertilization, and culture *in vitro*. Molecular Reproduction and Development. 37(1): 48-53.

Looney, C. R., B. R. Lindsey, C. L. Gonseth and Johnson, D. L. 1994. Commercial aspects of oocyte retrieval and *in vitro* fertilization (IVF) for embryo production in problem cows. Theriogenology. 41(1): 67-72.

Manik, R. S., S. K. Singla and Palta, P. 2003. Collection of oocytes through transvaginal ultrasound guided aspiration of follicles in an Indian breed of cattle. Animal Reproduction Science. 76 (3): 155-161.

Merton, J.S., A.P.W. De Roos, E. Mullaart, L. De Ruigh, L. Kaal, P.L.A.M. Vos and Dieleman, S.J. 2003. Factors affecting oocyte quality and quantity in commercial application of embryo technologies in the cattle breeding industry. Theriogenology. 59(2): 651-674.

Moore, K., C.J. Rodríguez-Sallaberry, J.M. Kramer, S. Johnson, E. Wroclawska, S. Goicoa and Niasari-Naslaji, A. 2007. *In vitro* production of bovine embryos in medium supplemented with a serum replacer: effects on blastocyst development, cryotolerance and survival to term. Theriogenology. 68(9):1316-1325.

Nedambale, T.L., A. Dinnyes, W. Groen, J.R. Dobrinsky, X.C. Tian and Yang, X. 2004. Comparison on *in vitro* fertilized bovine embryos cultured in KSOM or SOF and cryopreserved by slow freezing or vitrification. Theriogenology. 62(4): 437-449.

Oliveira, L.H., P.S. Carlos, S.S. Adriano, B.V. Marcio, A.L. Flavio, L.J.M. Jr. Pedro, C.W. Milo and Sartori, R. 2016. Follicle superstimulation before ovum pick-up for *in vitro* embryo production in Holstein cows. Journal Dairy Science. 99: 9307–9312.

Parrish, J. J., J. L. Susko-Parrish, E. S.Kritser, W. H. Eyestone and First, N.L. 1986. Bovine *in vitro* fertilization with frozen thawed semen. Theriogenology. 25: 591-600.

Pieterse, M.C., P.L.A.M. Vos, T.A. Kruip, Y.A. Wurth, T.H. Van Beneden, A.H. Willemse, and Taverne, M.A.M. 1991. Transvaginal ultrasound guided follicular aspiration of bovine oocytes. Theriogenology. 35(4)**:** 857-862.

Pontes, J. H. F., F. M. Sterza, A. C. Basso, C. R. Ferreira, B. V. Sanches, K. C. P. Rubin and Seneda, M. M. 2011. Ovum pick-up, *in vitro* embryo production, and pregnancy rates from a large-scale commercial program using Nelore cattle (Bos indicus) donors. Theriogenology. 75(9): 1640-1646.

Reis, A., M. E. Staines, R. G. Watt, D. F. Dolman and McEvoy, T.G. 2002. Embryo production using defined oocyte maturation and zygote culture media following repeated ovum pick-up (OPU) from FSH-stimulated simmental

heifers. Animal Reproduction Science. 72(4): 137-151.

Rizos, D., F. Ward, P. A. T. Duffy, M. P. Boland and Lonergan, P. 2002. Consequences of bovine oocyte maturation, fertilization or early embryo development *in vitro* versus *in vivo*: implications for blastocyst yield and blastocyst quality. Molecular Reproduction and Development. 61(2): 234-248.

Sirard, M.A., F. Richard, P. Blondin and Robert, C. 2006. Contribution of the oocyte to embryo quality. Theriogenology. 65(1): 126-136.

Summers, M.C., 2013. A brief history of the development of the KSOM family of media. Journal of Assisted Reproduction and Genetics, 30:995–999.

Tervit, H.R., D.G. Whittingham and Rowson, L.E.A. 1972. Successful culture *in vitro* of sheep and cattle ova. Journal Reproduction and Fertility, 30: 493-497.

Thompson, J.G., M. Mitchell and Kind, K.L. 2007. Embryo culture and long-term consequences. Reproduction Fertility and Development, 19(1): 43-52.

Totey, S. M., Pawshe , C. H. and Singh, G. P. 1993. *In vitro* maturation and fertilization of buffalo oocytes (*Bubalus bubalis*): Effects of media, hormones, and sera. Theriogenology. 39: 1153-1171.

Ward, F.A., P. Lonergan, B.P. Enright and M.P. Boland, 2000. Factors affecting recovery and quality of oocytes for bovine embryo production *in vitro* using ovum pick-up technology. Theriogenology. 54 (3): 433-446.

Wolfenson, D., Z. Roth and Meidan, R. 2000. Impaired reproduction in heat-stressed cattle: basic and applied aspects. Animal Reproduction Science, 60: 535-547.

Zicarelli, L., G. Neglia, V.C.D. Brienza, G. Papaccio, L. Esposito and Gasparrini, B. 2003. Buffalo (Bubalus bubalis) *in vitro* embryo production in two different defined culture media. Italian Journal of Animal Science. 2:136-138.

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