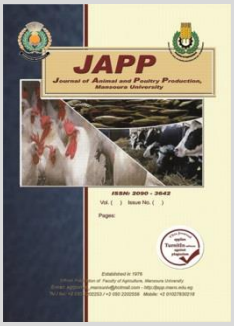
Journal of Animal and Poultry Production

Journal homepage & Available online at: www.jappmu.journals.ekb.eg

Effect of Culture Media, Gonadotropins, Proteins, Growth Factors and Hyaluronic Acid on *In-Vitro* Maturation of Buffalo Oocytes

Khadr, A. H.[1]; A. Abdou [1]; A. M. El-Sherbiny[1] and A. S. S. Abdoon[2]*

[1]Animal Prod. Dept., Faculty Agric., Ain Shams University, Cairo, Egypt.
[2]Department of Animal Reproduction and Artificial Insemination, Veterinary Research Institute, National Research Center, Cairo, Egypt.



ABSTRACT

This study was carried out to investigate the effect of culture media, gonadotropins, proteins, growth factors and hyaluronic acid (HA) on the IVM rate of buffalo oocytes. Cumulus oocyte complexes were aspirated from ovaries and classified into 4 Grades, excellent and good quality COCs. Oocyte maturation was tested using TCM199, CR1aa or DMEM (Experiment 1); 10 µg/mL FSH + 50 IU/mL eCG, 10µg/mL FSH or 50 IU/mL eCG (Experiment 2): 10% FCS, 4mg/ml BSA or 1 µg/mL PVA (Experiment 3). Control group;10 µg/mL IGF-1 or 10 µg/mL EGF (Experiment 4) or 0, 2.5, 5 and 10 µg HA (Experiment 5). In all experiments, COCs were cultured for 252-24 h, then cytoplasmic and nuclear maturation was conducted. Results indicated that TCM199 achieved the best cytoplasmic and nuclear maturation. TCM199 supplemented with FSH and/or eCG enhanced *in-vitro* cytoplasmic and nuclear maturation of buffalo oocytes. Adding FCS, using growth factors, or 2.5 µg/mL HA toTCM199 lead to improving maturation rate. the results indicated that in-vitro maturation of buffalo oocytes is improved in TCM-199 supplemented with 50 IU eCG, 10µg/mL FSH, 10% FCS, 10 µg/ml EGF or 2.5 µg/mL HA.

***Keywords*:** Culture media; Gonadotropins; Protein supplement; Growth factors; Hyaluronic acid.

INTRODUCTION

Buffalo is the backbone of animal resources as the primary source of meat and milk in Egypt (Fahim *et al*. 2018). However, the reproductive efficiency of buffaloes decreased due to management and biological factors. Assisted reproductive technologies are a promising solution (Warriach *et al*. 2015). Despite this, the techniques for producing *in-vitro* buffalo embryos still require more effort (Marin *et al*. 2019).

Producing matured oocytes with good quality and quantity is an important criterion for fertilization and blastocyst rate (Widayati and Pangestu, 2020). *In-vitro* oocyte maturation didn`t mimic the same environment as *in-vivo*, and that caused reducing the quality of oocytes (Abd El-Aziz *et al*. 2016). This may be due to the many factors affecting *in-vitro* maturation, such as season of collection, ovarian status, follicle size, oocyte quality, method of recovery, etc. Culture media is one of the important factors that affect oocyte maturation rate and the quality of matured oocytes (Pereira *et al*. 2019). Tissue culture medium 199 (TCM-199) is widely spread among all IVM labs. (Zhao *et al*. 2009). whereas Christopher Rosenkranz's 1aa (CR1aa) culture media was developed for using in-vitro maturation, fertilization, and culture, in addition, it has a simple composition, which makes it easy to produce in large amounts at low cost (Somfai *et al*. 2010). Adding hormones to IVM media leads to improve maturation rate (Abbasi and Sadrkhanlou, 2000). Basic maturation media of most mammalian oocytes supplemented with follicle stimulating hormone (FSH) is expensive compared with equine chorionic gonadotropin (eCG) (Kouamo and Kharche, 2017). The serum is one of the important elements of IVM media (Chandra *et al*. 2011, Sreenivas *et al*. 2014). Fetal calf serum (FCS) contains endotoxins that can inconsistently emerge with oocyte maturation rate and *in-vitro* embryonic development (McKiernan and Bavister, 1992). The global demand for FCS has increased (Sreenivas *et al*. 2014), and there is a variation between serum batches (Verma *et al*. 2014). Not forgetting that some FCS are toxic and may be contaminated with fungi, viruses, and mycoplasma (Evans 2014). Replacement of mammal's serum with other components such as polyvinyl alcohol (PVA) or bovine serum albumin (BSA) *in-vitro* embryo culture media to treat the problems of serum was a challenge in IVF laps (Watson *et al*. 2000, Del Collado *et al*. 2014 Quan *et al*. 2017). Growth factors promoted oocyte maturation in many species (Arat *et al*. 2016). Hyaluronic acid (HA) is the major glycosaminoglycans (GAGs) found in follicular and oviduct fluid (Ríos *et al*. 2015). It is produced by cumulus cells during oocyte maturation, it is important for cumulus cell expansion, nuclear maturation, and embryo development. The HA concentration is a critical factor in the success IVM process (Opiela *et al*. 2014).

In-vitro maturation technique developed for buffalo oocytes has tremendously been progressed over decades, but many factors such as maturation media, hormones, supplemented protein, growth factors, HA, oocytes quality, incubation condition, etc. are still influencing their competence and still need to be investigated. Therefore, the focus has been on some factors affecting IVM of buffalo oocytes to obtain a suitable maturation media. This research

* **Corresponding author.**
E-mail address: assabdoon@yahoo.com / as.abdoon@nrc.sci.eg
DOI: 10.21608/jappmu.2022.145223.1043

aims to examine the effect of the type of IVM medium, supplementation of IVM media with hormones, different protein sources, growth factors, and HAon buffalo oocytes' maturation rate.

MATERIALS AND METHODS

Collection of ovaries

This work was executed at the Department of Animal Reproduction and Artificial Insemination, Veterinary Research Institute, National Research Center. For five experiments, buffalo ovaries were collected from a local slaughterhouse (Shubra El-Khaimah, Qualyubia, Egypt) immediately after slaughter, and relocated to the lab in warmed 0.9% sodium chloride salt solution within 2 hours. In Lap, ovaries were rinsed with warmed saline solution three times and once with 70% ethel alcohol. Cumulus-oocytes complexes (COCs) were aspirated from non-atretic antral follicles (4–8 mm diameter) by a sterilized disposal syringe connected to an 18-gauge needle packed with 2 mL phosphate buffer saline (PBS). Aspirated COCs with follicular fluids were pooled in a sterile 15 mL falcon tube and left in water-bass at 37°C for 15 min to assemble the oocytes at the tube bottom. Then, the sediments were aspirated and put in a 10 cm aseptic Petri dish and PBS was mixed with sediments then, examined under a stereomicroscope and rinsed 3 times in PBS and once in culture medium. then COCs was classified into 4 categories as excellent, good, fair, and denuded according to the method described previously (Abdoon *et al*. 2018).

Experimental design

Five experiments were conducted according to the following experimental design:

Exper.	Factors studied	Variable	Additives/medium*
1	Three types of maturation media	1) TCM-199 2) CR1aa 3) DMEM	10% FCS, 50 IU/mL eCG and 10 µg/mL FSH for each medium.
2	Gonadotropin hormones	1) 10 µg/mL FSH+50 IU/mL eCG 2) 10 µg/mL FSH 3) 50 IU/mL eCG	TCM-199 with 10% FCS.
3	Protein supplementation	1) 10% FCS 2) 4 mg/mL BSA 3) 1 µg/mL PVA	TCM-199 with 50 IU/mL eCG and 10 µg/mL FSH.
4	Growth factors	1) Control 2) 10 µg/mL IGF-1 3) 10 µg/mL EGF	TCM-199 with 10% FCS, 50 IU/mL eCG, and 10 µg/mL FSH.
5	Hyaluronic acid (HA) level	1) Control 2) 2.5 µg HA/ mL 3) 5.0 µg HA/ mL 4) 10 µg HA/ mL	TCM-199 with 10% FCS, 10 µg/mL FSH, and 50 IU/mL eCG.

*** All media used were supplemented with antibiotics (50 µg/mL gentamycin) DMEM: Dulbecco's Modified Eagle low glucose medium.**

In all experiments, COCs were matured in 0.5 mL of maturation medium in a four-well culture dish (Nunclon, Denmark) in a CO2 incubator for 22 to 24 hours at 38.5˚C in humidified air of 5% CO_2.

The maturation rate was determined after 22-24h of IVM. Oocyte's maturation was judged according to The nuclear maturation as indicated by absence (Fig. 1A) or presence of the 1st polar body (Figure 1B), and cytoplasmic maturation by determining the degree of cumulus-cell expansion as follows: grade zero (G0): no cumulus-cell expansion, grade one (G1): with slight cumulus-cell expansion in the outer layer of cumulus-cells, grade two (G2): with moderate cumulus-cell expansion, grade three (G3): with full cumulus-cell expansion (Figure 2).

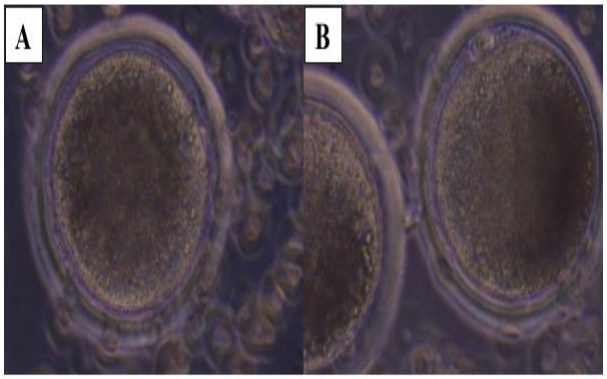


Figure 1. IVM buffalo oocytes showed absence of 1st polar body (A) and presence of 1st polar body (B).

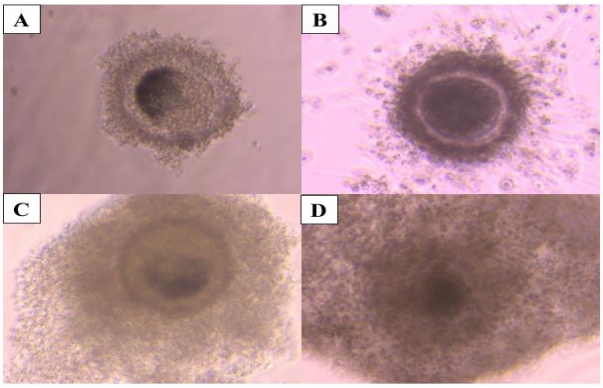


Figure 2. Showed grade zero (A), grade 1(B), grade 2 (C) and grade 3 (D) of cumulus- cells expansion (magnification power 40X).

Statistical Analysis

The percentages of oocyte maturation in different experiments were compared using one-way analyses of variance by the SAS program (SAS, 2011). Variations between the different empirical groups were examined by Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Experiment 1:

Influence of culture media on maturation rate of buffalo oocytes.

Results indicated that buffalo oocytes incubated in TCM199 show significantly higher percentage of both

cytoplasmic in term of the highest oocytes at G3 and nuclear maturation rate ((P< 0.05) followed by that incubated in CR1aa, while DMEM medium showed the lowest values (Table 1).

Table 1. Percentage of buffalo oocytes at different grades of cytoplasmic maturation and nuclear maturation rate (mean±SE) as effected by type of maturation media.

Media	Classification of cytoplasmic maturation (%)				Nuclear MR
	G3	G2	G1	G0	1st PB extruded
TCM199	52.27[a] ±1.15	17.13 ±0.51	14.62[a] ±4.28	15.99[c] ±3.66	79.28[a] ±1.75
CR1aa	44.75[b] ±1.26	20.50 ±1.29	12.50[a] ±0.58	22.25[b] ±1.26	73.50[b] ±1.29
DMEM	40.50[c] ±1.29	20.00 ±2.83	8.25[b] ±1.50	31.25[a] ±1.26	55.00[c] ±4.40

#Overall means within a column with different superscript letters differ significantly (P< 0.05).

***G0: no cumulus expansion, G1 with slight expansion of the outer layer of cumulus cells. G2 with moderate cumulus cell expansion, and G3 with full cumulus-cell expansion.**

- PB: Polar body, MR: maturation rate

The study found that TCM199 significantly improved the maturation rate of IVM buffalo oocytes compared with CR1aa or DMEM. A similar result was previously reported (Nowshari, 2005, Kandil *et al*. 2014). The superiority of TCM199 in *in-vitro* the maturation of buffalo oocytes may be due to the vitamins found in this medium. On the other hand, Hemeida *et al*. (2015) found a slight increase in percentage of camel oocytes matured in CR1aa compared with TCM199, while Abdoon *et al*. (2014b) found no considerable difference in the maturation rate of Jennie's oocytes matured in TCM 199 or CR1aa. Other results showed that the oocytes maturation rate was better using DMEM than TCM199 in the bovine (Smetanina *et al*. 2000, Kumar *et al*. 2015) in goat and in Jennies oocytes (Abdoon *et al*. 2014a). While Rungsiwiwut *et al*. (2005) reported no significant differences between TCM199 and DMEM on domestic cat oocytes maturation This discrepancy might be related to species differences or differences in cultural conditions.

Experiment 2:

Effect of supplementing tcm199 with gonadotropin hormones on maturation rate of buffalo oocytes.

The impact of supplementing TCM199 with FSH and/or eCG on cytoplasmic and nuclear maturation on buffalo oocytes are shown in Table 2.

Table 2. Effect of supplementing maturation medium with gonadotropin hormones on cytoplasmic and nuclear maturation of buffalo oocytes.

Media	Classification of cytoplasmic maturation (%)				Nuclear MR
	G3	G2	G1	G0	1st PB extruded
FSH + eCG	52.67[a] ±0.58	17.33[a] ±0.58	15.00 ±4.58	15.00[b] ±4.00	80.33[a] ±1.53
FSH	45.00[b] ±2.00	12.00[b] ±1.00	18.67 ±2.08	24.33[a] ±1.15	69.67[b] ±2.08
eCG	51.98[a] ±0.97	13.57[b] ±0.98	13.92 ±3.53	20.53[ab] ±2.50	77.67[a] ±2.52

#Overall means within a column with different superscript letters differ significantly (P< 0.05).

***G0: no cumulus expansion, G1 with slight expansion of the outer layer of cumulus cells. G2 with moderate cumulus cell expansion, and G3 with full cumulus-cell expansion.**

- PB: Polar body, MR: maturation rate

Supplementation of TCM199 with eCG with or without FSH achieved significant (P<0.05) increase of full cumulus-cell expansion (G3) percentage and percentage of 1st polar body (Cytoplasmic and nuclear maturation). Regarding the percentage of moderate cumulus-cell expansion (G2). IVM medium supported with combination of FSH and eCG showed significantly (P<0.05) the highest percentage compared with IVM medium with FSH or eCG alone. On the other hand, there is no significant (P>0.05) difference between slight cumulus cell expansions (G1) percentage of buffalo oocytes cultured in IVM medium provided with FSH with or without eCG. Addition of FSH to IVM medium lead significantly (P<0.05) to higher percentage of G0 compared with adding eCG with FSH.

It was reported that FSH stimulates the production of cAMP from cumulus cells to assist the oocyte enter meiotic maturation (Jaffe and Egbert, 2017). Widayati and Pangestu (2020) reported that provide maturation medium with FSH enhanced in-vitro oocyte maturation. Addition of FSH to culture media was important to induce the nuclear and cytoplasmic maturation, Although, FSH is necessary to spontaneous oocyte maturation, it is commonly believed that these hormones enhance oocyte cytoplasmic maturation significantly by adjusting the cumulus cell activities (Da Broi *et al*. 2018, Tetkova *et al*. 2019). In study on buffalo oocytes Hegab *et al*. (2009) found that maturation rate was 80.4% and 73.8% in TCM199 supplemented with 50 µg/mL eCG and 50 µg/mL FSH respectively. Supplementing the maturation media with FSH or eCG enhances subsequent buffalo embryo development stages (Abdoon *et al*. 2001).

Experiment 3:

Effect of adding protein or PVA to maturation media on maturation rate of buffalo oocytes.

The influence of adding FCS, BSA or PVA to maturation medium on cytoplasmic and nuclear maturation of buffalo oocytes are shown in Table 3. Adding FCS as a supplement to IVM medium improved (P< 0.05) the percentage of cytoplasmic and nuclear maturation rate of buffalo oocytes when compared with BSA or PVA. However, there is no significant (P>0.05) difference between moderate cumulus cell (G2) expansion percentage of buffalo oocytes cultured in IVM medium supplemented with FCS, BSA and PVA. Whereas, adding FCS or BSA to IVM medium significantly increased percentage of slight cumulus-cell expansion (G1) compared with adding PVA to maturation medium.

Protein presence in IVM media have powerful effect on embryo development (Ali and Sirard, 2002). Also, it has an important role in acceleration formation of pronuclei (Eckert and Niemann, 1995). Albumin chelates heavy metals and participate in pH buffering (Mehta and Kiessling, 1990). Also, protein can inhibit the effect of reactive oxygen species (Natsuyama *et al*. 1993).

Adding FCS to maturation media enhance oocytes maturation compare with BSA in buffalo (Deneke *et al*. 2013). Similar results were reported in cattle (Sreenivas *et al*. 2014; del Collado *et al*. 2015), This may be due to the abundance of nutrients, growth factors, antioxidants in FCS (Sreenivas *et al*. 2014; Puri *et al*. 2015). Also, its high content of lipid compared to BSA (del Collado *et al*. 2015).

This would provide oocyte with energy for growth and development (Prates *et al*. 2014).

Table 3. Effect of adding protein or PVA to maturation media on cytoplasmic and nuclear maturation of buffalo oocytes.

Media	Classification of cytoplasmic maturation (%)				Nuclear MR
	G3	G2	G1	G0	1st PB extruded
FCS	52.67[a] ±0.58	17.33 ±0.58	15.00[b] ±4.58	15.00[c] ±4.00	80.33[a] ±1.53
BSA	47.67[b] ±2.08	14.67 ±4.93	10.33[b] ±2.08	27.33[b] ±2.08	70.00[b] ±2.65
PVA	27.00[c] ±2.65	13.67 ±1.53	19.00[a] ±2.65	40.33[a] ±2.52	48.00[c] ±6.24

#Overall means within a column with different superscript letters differ significantly (P< 0.05).

***G0: no cumulus expansion, G1 with slight expansion of the outer layer of cumulus cells. G2 with moderate cumulus cell expansion, and G3 with full cumulus-cell expansion.**

- PB: Polar body, MR: maturation rate

Experiment 4:

Effect of adding growth factors to maturation medium on maturation rate of buffalo oocytes.

The influence of adding IGF-1 or EGF to maturation medium on cytoplasmic and nuclear maturation of buffalo oocytes are shown in Table 4. Enrichment IVM (TCM-199) medium with EGF increased (P<0.05) percentage of G3 and 1st polar body extruded when compared with IGF-1 or control. while there is no significant (P>0.05) difference between moderate cumulus cell expansion (G2) percentage of buffalo oocytes cultured in IVM medium supplemented with or without growth factor. Regarding the percentage of slight cumulus-cell expansion (G1) IVM medium without addition obtained high (P<0.05) percentage compared with IVM medium supported with EGF, there is no significant difference when adding IGF-1 to IVM medium than controlled maturation medium or maturation medium with EGF. Controlled maturation medium or maturation medium supplemented with IGF-1 increased (P<0.05) percentage of G0 compared to add EGF to maturation medium.

Table 4. Effect of adding growth factors to maturation media on cytoplasmic and nuclear maturation of buffalo oocytes.

Media	Classification of cytoplasmic maturation (%)				Nuclear MR
	G3	G2	G1	G0	1st PB extruded
Control	52.67[b] ±0.58	17.33 ±0.58	15.00[a] ±4.58	15.00[a] ±4.00	80.33[b] ±1.53
IGF-1	53.67[b] ±3.21	20.67 ±7.02	13.67[ab] ±2.89	12.00[a] ±2.00	84.67[a] ±2.52
EGF	61.67[a] ±2.31	26.00 ±2.00	7.00[b] ±2.65	5.33[b] ±0.58	88.00[a] ±1.73

#Overall means within a column with different superscript letters differ significantly (P< 0.05).

***G0: no cumulus expansion, G1 with slight expansion of the outer layer of cumulus cells. G2 with moderate cumulus cell expansion, and G3 with full cumulus-cell expansion.**

- PB: Polar body, MR: maturation rate

Sirisathien *et al*. (2002) suggested that EGF act directly on oocytes and/or cumulus cell. Also, addition of EGF to maturation medium for sheep oocytes led to enhance cleaving rate and blastocyst formation (Kelly *et al*. 2008). However, Vinayak, (2018) showed that adding EGF to maturation medium can improve bovine oocyte maturation. Yousef *et al*. (2018) reported that addition of 10-20 ng/mL EGF to IVM medium promotes the maturation of buffalo oocytes. Also, Nagar and Purohit, (2005) mentioned that supplementing the IVM medium with 10 to 100 ng/mL of EGF increased oocytes maturation of goat. EGF is involved in regulating cell proliferation and apoptosis (Chen *et al*. 2017). IGF-1 and EGF play a valuable role in promoting oocyte maturation and embryo development in many mammalian species (Arat *et al*. 2016; Yang *et al*. 2019). In contrast, supplementation of IVM with IGF-1 improve *in-vitro* maturation in buffalo (Kumar and Purohit, 2004), in yak-cattle crossbred (Yang *et al*. 2019), in ovine (Kelly *et al*. 2008) in porcine (Mahanta *et al*. 2018) and in human (Yu *et al*. 2012). IGF-1 has multiple functions in cellular metabolism, growth, proliferation, and differentiation (Meiyu *et al*. 2015). Supplementing IVM medium with IGF-1 enhanced the developmental competence of sheep oocytes compared with control group (Javvaji *et al*. 2020). Also, Li *et al*. (2016) reported that enriching the IVM medium with IGF-1 to mouse COCs improve cumulus-cells expansion and oocyte nuclear maturation in mouse. This difference could be due to the dose or culture conditions

Experiment 5

Effect of adding different concentrations of hyaluronic acid to maturation medium on maturation rate of buffalo oocytes.

The influence of adding 2.5, 5.0 or 10 µg/mL hyaluronic acid (HA) to maturation medium on cytoplasmic and nuclear maturation of buffalo oocytes is presented in Table 5. The percentage of full cumulus-cells expansion and nuclear maturation rate was significantly the highest by adding 2.5µg/mL HA to maturation media compared with other groups, while increasing level of HA to 10µg/mL showed the lowest values as compared to control and other levels of HA.

Table 5. Effect of adding different concentrations of hyaluronic acid (HA) to maturation medium on cytoplasmic and nuclear maturation of buffalo oocytes.

Media	Classification of cytoplasmic maturation (%)				Nuclear MR
	G3	G2	G1	G0	1st PB extruded
Control	52.67[b] ±0.58	17.33[c] ±0.58	15.00[ab] ±4.58	15.00[b] ±4.00	80.33[b] ±1.53
10 µg/mL HA	25.00[d] ±2.65	20.67[b] ±3.06	19.67[a] ±5.13	34.67[a] ±1.15	44.67[c] ±3.51
5 µg/mL HA	40.33[c] ±2.52	28.67[a] ±1.15	19.33[a] ±1.15	11.67[bc] ±2.08	81.67[b] ±1.53
2.5 µg/mL HA	57.67[a] ±2.52	22.67[b] ±1.53	12.00[b] ±1.73	7.67[c] ±2.52	89.33[a] ±2.08

#Overall means within a column with different superscript letters differ significantly (P< 0.05).

***G0: no cumulus expansion, G1 with slight expansion of the outer layer of cumulus cells. G2 with moderate cumulus cell expansion, and G3 with full cumulus-cell expansion.**

- PB: Polar body, MR: maturation rate

Cumulus-cells expansion is necessary for oocyte maturation (Yokoo *et al*. 2007) And HA concentration is a critical factor in success IVM process (Opiela *et al*. 2014).Suppression of HA synthesis during oocyte maturation led to significantly inhibited cumulus-cells expansion (Yokoo *et al*. 2008) In accordance with the present results, Marei *et al*., (2012) suggested that low

concentration of HA stimulate oocytes to enter in nuclear maturation. Based on these knowledge's, it was supposed that the addition of exogenous HA with an adequate concentration could enhance the percentage of oocytes maturation. In this respect, Opiela *et al.* (2014) found that using oocyte maturation medium containing 0.375 mg/mL or 0.750 mg/mL HA has no significant effect on oocyte nuclear maturation. Also, Opiela *et al*. (2014) and Corn *et al*. (2005) mentioned that increasing concentration of HA above 0.07% during IVM may be risky on oocyte maturation. However Marei *et al*. (2012) showed no significant influence of addition 0, 0.1, 0.5 or 1 mg/mL of HA in maturation media on percentage of bovine oocytes matured.

CONCLUSION

The results indicated that *in-vitro* maturation of buffalo oocytes is improved in TCM-199 supplemented with 50 IU eCG, 10µg/mL FSH , 10% FCS , 10 µg / ml EGF or 2.5 µg /mL HA.

REFERENCES

Abbasi, M. and Sadrkhanlou, R.A. (2000). In Vitro Fertilization And Early Embryonic Development In Buffalo. Journal of Veterinary Research. 55, 33-36.

Abd El-Aziz, A.H., Mahrous, U.E., Kamel, S.Z. and Sabek, A. A. (2016). Factors Influencing in vitro Production of Bovine Embryos: A Review Asian Journal of Animal and Veterinary Advances. ISSN 1683-9919. DOI: 10.3923/ajava.2016.737.756

Abdoon, A., Abdel-Rahman, H., Shawky, S., Kandil, O. and Fathalla, S. (2014a). Influence of follicle size, methods of retrieval on oocytes yield and morphology in Egyptian Jennies ovaries with special reference to maturation rate in vitro. Veterinary research communications. 38, 287–295. DOI 10.1007/s11259-014-9617-y

Abdoon, A., Fathalla, S., Shawky, S., Kandil, O., Kishta, A. and Masoud, S. (2018). In Vitro Maturation and Fertilization of Donkey Oocytes. Journal of Equine Veterinary Science. 65, 118-122. DOI: 10 .1016 / j.jevs.2018.04.007.

Abdoon, A., Gabler, C., Holder, C., Kandil, O. and Einspanier, R. (2014b). Seasonal variations in developmental competence and relative abundance of gene transcripts in buffalo (Bubalus bubalis) oocytes. Theriogenology. 82, 1055-1067. doi: 10.1016/j.theriogenology.2014.07.008

Abdoon, A., Kandil, O., Otoi, T. and Suzuki, T. (2001). Influence of oocyte quality, culture media and gonadotropins on cleavage rate and development of in vitro fertilized buffalo embryos. Anim. Reprod. Sci. 65, 215-223.

Ali, A. and Sirard, M. (2002). Effect of the Absence or Presence of Various Protein Supplements on Further Development of Bovine Oocytes During In Vitro Maturation. Biology of reproduction. 66, 901-905.

Arat, S., Caputcu, A.T. , Cevik, M., Akkoc, T., Cetinkaya, G. and Bagis, H. (2016). Effect of growth factors on oocyte maturation and allocations of inner cell mass and trophectoderm cells of cloned bovine embryos. Zygote. 24 (4), 554-562. Doi :10. 1017 / S0967199415000519.

Chandra V., Kumar, G.S. and Sharma, G.T. (2011). Temporal expression pattern of insulin-like growth factors (IGF-1 and IGF-2) ligands and their receptors (IGF-1R and IGF-2R) in buffalo (Bubalus bubalis) embryos produced in vitro. Livestock Science. 135, 225–230. doi:10.1016/j.livsci.2010.07.016

Chen , Pan, Y, Cui, Y, Wen, Z, Liu, P, He, P, Li, Q, Peng, X, Zhao, T. and Yu, S. (2017). Insulin-like growth factor I enhances the developmental competence of yak embryos by modulating aquaporin. Reprod. Domest. Anim. 52, 825-835. DOI: 10. 1111 /rda .12985

Corn, C., Hauser-Kronberger, C., Moser, M., Tews, G. and Ebner T. (2005). Predictive value of cumulus cell apoptosis with regard to blastocyst development of corresponding gametes. Fertil. Steril. 84, 627-633. DOI: 10.1016/j.fertnstert.2005.03.061

Da Broi, M., Giorgi, V., Wang, F., Keefe, D., Albertini, D. and Navarro, P.. (2018). Influence of follicular fluid and cumulus cells on oocyte quality: clinical implications. Journal of Assisted Reproduction and Genetics. 35, 735–751. doi: 10.1007/s10815-018-1143-3

Del Collado, M., Saraiva, N., Lopes, F., Gaspar, R., Padilha L., Costa, R., Rossi, G., Vantini, R. and Garcia, J. (2015). Influence of bovine serum albumin and fetal bovine serum supplementation during in vitro maturation on lipid and mitochondrial behaviour in oocytes and lipid accumulation in bovine embryos. Reproduction, fertility, and development. 28, 1721–1732. DOI: 10.1071/RD15067

Del Collado, M., Saraiva, N.Z., Lopes, F.L., Cruz, M.H., Gaspar, R.C., Oliveira, C.S., Perecin, F. and Garcia, J.M. (2014). Effects of reduction or replacement of fetal calf serum by other compounds during in vitro maturation of bovine oocytes. Pesq. Vet. Bras. 34(7), 689-694.

Deneke, Y., Yadav, P., Deb, R. and Nanda, T. (2013). Comparative studies of the effect of BSA vs FCS as a supplement in TCM-199 on the in vitro maturation rate of buffalo oocytes collected from slaughterhouse ovaries. Buffalo Bulletin. 32, 21-25.

Duncan, D.B. (1955). Multiple range and multiple F-test. Biometrics. 11, 1-42.

Eckert, J. and Niemann, H. (1995). In vitro maturation, fertilization and culture to blastocysts of bovine oocytes in protein-free media. Theriogenology. 43, 1211-1225.

Evans M (2014) Isolation and Maintenance of Murine Embryonic Stem Cells, Essentials of Stem Cell Biology In: Lanza R, Atala A, (Ed), Essentials of Stem Cell Biology (Third Edition), Academic Press, pp 399-408, DOI 10.1016/B978-0-12-409503-8.00028-7.

Evans M (2014) Isolation and Maintenance of Murine Embryonic Stem Cells, Essentials of Stem Cell Biology In: Lanza R, Atala A, (Ed), Essentials of Stem Cell Biology (Third Edition), Academic Press, pp 399-408, DOI 10.1016/B978-0-12-409503-8.00028-7.

Fahim, N.H., Abdel-Salam, S.A., Mekkawy, W., Ismael, A., Abou-Bakr, S., El Sayed, M and Ibrahim, M.A. (2018). Delta and upper Egypt buffalo farming systems: a survey comparison. Egyptian J. Anim. Prod. 55(2), 95-106.

Hegab, A.O., Montasser, A.E., Hammam, A.M., Abu El-Naga, E. and Zaabel, S. (2009). Improving in vitro maturation and cleavage rates of buffalo oocytes. Anim. Reprod. 6, 416-421.

Hemeida, N.A., Abd-Elfatah, A., Elsayed, M.A. and Kandil, O. (2015). Effect of culture media and epidermal growth factor on in vitro oocyte maturation in the one-humped camel (Camelus dromedarius). International Journal of ChemTech Research. 8 (12), 130-141.

Jaffe, L. and Egbert, J. (2017). Regulation of Mammalian Oocyte Meiosis by Intercellular Communication Within the Ovarian Follicle. Annual review of physiology. 79, 237-260. DOI: 10.1146/annurev-physiol-022516-034102

Javvaji, P., Dhali, A., Rabinson, J., Kolte, A., Sellappan, S., Mech, A. and Sejian, V. (2020). IGF-1 treatment during in vitro maturation improves developmental potential of ovine oocytes through the regulation of PI3K/Akt and apoptosis signaling. Animal Biotechnology. 32, 1-8. DOI:10.1080/10495398.2020.1752703

Kandil, O., M. El-Nahla, A., Siam, A.A., Abdoon, A.S.S., Al-Monifi, F.A. and Mermillod, P. (2014). Effect of Media on In vitro Maturation Rate of Dromedary Camel Oocytes Global Veterinaria. 13 (2), 159-165.

Kelly, J., Kleemann, D., Maxwell, W. and S. Walker. (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, 570-578.

Kouamo, j. and Kharche, S.D. (2017). FSH and LH can be substituted by eCG in maturation media for caprine embryo or parthenote production in vitro. 7th International Veterinary Congress J. Vet. Sci. Technol. 8 (4). DOI: 10.4172/2157-7579-C1-025.

Kumar, D. and Purohit, G.N. (2004). Effect of epidermal and insulin-like growth factor-1 on cumulus expansion, nuclear maturation and fertilization of buffalo cumulus oocyte complexes in simple serum free media DMEM and Ham's F-10. Veterinarski Arhiv. 74, 13-25.

Kumar, S., Gedam, S., Biswas, R.K., Purkayastha, A., Devi, B., Bharti, P., Doley, S. and Kadirvel, G. (2015). Effect of hormones, follicular fluid, serum and media on in vitro maturation of porcine oocyte. Indian Journal of Animal Sciences. 85, 958-961.

Li, Y., Liu, Q., Chen, Q., Yan, X. and Li, N. (2016). Insulin-like growth factor 1 promotes cumulus cell expansion and nuclear maturation of oocytes via Pi3K/Akt pathway. Int. J. Clin. Exp. Pathol. 9, 11436-11443 .

Mahanta, N., Bhuyan, D., Kumar, S., Biswas, R., Dutta, D., Deka, B.C., Das, A., Dewry, R. and Hazarika, G. (2018). Effect of growth factor on in-vitro maturation of porcine oocyte. Indian Journal of Animal Research. 52, 353-357. DOI: 10.18805/ijar.B-3202

Marei, W.F., Ghafari, F. and Fouladi-Nashta, A.A. (2012). Role of hyaluronic acid in maturation and further early embryo development of bovine oocytes. Theriogenology. 78 (3), 670-677. doi: 10.1016 /j. theriogenology.2012.03.013

Marin, D.F.D., de Souza, E.B., de Brito, V.C., Nascimento, C.V., Ramos, A.S., Filho, S.T.R., da Costa, N.N., Cordeiro, M.d., Santos,, S.D.D. and Ohashi, O.M. (2019). In vitro embryo production in buffaloes: from the laboratory to the farm. Anim. Reprod. 16 (2), 260-266. DOI: 10.21451/1984-3143-AR2018-0135

McKiernan, S.H. and Bavister, B.D. (1992) Different lots of bovine serum albumin inhibit or stimulate in vitro development of hamster embryos. In Vitro Cell Dev. Biol. 3 (1),154-166. doi: 10.1007/BF02631084.

Mehta, T. and Kiessling, A. (1990). Developmental potential of mouse embryos conceived in vitro and cultured in EDTA with or without amino acids or serum. Biology of reproduction. 43, 600-606.

Meiyu, Q., Liu, D. and Roth, Z. (2015). IGF-I slightly improves nuclear maturation and cleavage rate of bovine oocytes exposed to acute heat shock in vitro. Zygote. 23 (4), 514-524. DOI: 10 .1017 /S096719941400015X

Nagar, D. and Purohit, G.N. (2005). Effect of epidermal growth factor on maturation and fertilization in vitro of goat follicular oocytes in a serum free or serum supplemented medium. Veterinarski Arhiv. 75, 459-467.

Natsuyama, S., Noda, Y., Narimoto, K. and Mori, T. (1993). Role of protein supplements in the culture of mouse embryo. Theriogenology. 40, 149-157.

Nowshari M. (2005). The effect of harvesting technique on efficiency of oocyte collection and different maturation media on the nuclear maturation of oocytes in camels (Camelus dromedarius). Theriogenology. 63 (9), 2471-2481.

Opiela, J., Romanek, J., Lipiński, D. and Smorąg, Z. (2014). Effect of Hyaluronan on Developmental Competence and Quality of Oocytes and Obtained Blastocysts from In Vitro Maturation of Bovine Oocytes. BioMed research international. 2014:519189. doi: 10.1155/2014/519189

Pereira, B., Zangeronimo, M., Castillo, M., Gadani, B., Chaves, B., Rodríguez-Gil, J., Bonet, S. and Yeste, M. (2019). Supplementing Maturation Medium With Insulin Growth Factor I and Vitrification-Warming Solutions With Reduced Glutathione Enhances Survival Rates and Development Ability of in vitro Matured Vitrified-Warmed Pig Oocytes. Frontiers in Physiology. 9, 1894-1907. doi: 10.3389/fphys.2018.01894.

Prates E.G., Nunes, J.T. and Pereira, R.M. (2014). A Role of Lipid Metabolism during Cumulus-Oocyte Complex Maturation: Impact of Lipid Modulators to Improve. PMID: 24733963. PMCID : PMC3964899. DOI : 10.1155/2014/692067 .

Puri, G., Chaudhary, S., Singh, V. and Sharma, A. (2015). Effects of fetal bovine serum and estrus buffalo serum on maturation of buffalo (Bubalus bubalis) oocytes in vitro. Veterinary world. 8, 143-146. doi: 10.14202/vetworld.2015.143-146.

Quan, Guo Bo, Ma, Yuan, Ni, Yi Na, Wu, Guo Quan, Chun Rong, Hong and Qiong Hua (2017). Effects of synthetic polymers on in vitro maturation of sheep oocytes and subsequent developmental capacity after parthenogenetic activation or fertilization. Small Ruminant Research. 153, 153-157. doi: 10.1016/j.smallrumres.2017.06.008

Ríos, G.L., Buschiazzo, J., Mucci, N.C., Kaiser, G.G., Cesari, A. and Alberio, R.H. (2015). Combined epidermal growth factor and hyaluronic acid supplementation of in vitro maturation medium and its impact on bovine oocyte proteome and competence. Theriogenology. 83 (5), 874-880. doi: 10.1016/j.theriogenology.2014.11.022

Rungsiwiwut,R., Thongphakdee,A., Numchaisrika,P., Sirivaidayapong, S. and Techakumphu, M. (2005). The effect of culture media and culture time on the in vitro maturation of domestic cat oocytes. Thai. J. Vet. Med. 35, 39-45.

SAS (2011). Base SAS 9.3 Procedure Guide: Statistical Procedure. Cary, NC, US .

Sirisathien, S. (2003). Development and application of bovine in vitro fertilization. Doctor of philosophy. Athens, georgia.

Smetanina, I., Tatarinova, L. and Krivokharchenko, A. (2000). The effect of the composition of the culture media on bovine oocyte maturation and embryo development in vitro. Ontogenez. 31, 139-143.

Somfai, T., Inaba, Y., Aikawa, Y., Ohtake, M., Kobayashi, S., Konishi, K., Nagai, T. and Imai, K. (2010). Development of bovine embryos cultured in CR1aa and IVD101 media using different oxygen tensions and culture systems. Acta Veterinaria Hungarica. 58 (4), 465-474. DOI: 10.1556/AVet.58.2010.4.7

Sreenivas, D., Dowluru, K.S., Yarla, N.S., Thomas, V., PalniSamy, A., Vadlapudi, V. and Preethi, R. (2014). In Vitro Production of Sheep Embryos in CR1aa Medium Supplemented with L-Ascorbic Acid. tissue science and engineering. 5 (1), 131-138. doi:10.4172/2157-7552.1000131

Tetkova, A., Susor, A., Kubelka, M., Nemcova, L., Jansova, D., Dvoran, M., Del Llano, E., Holubcova, Z. and Kalous, J. (2019). Follicle-stimulating hormone administration affects amino acid metabolism in mammalian oocytes. Biology of reproduction. 101 (4), 719-732. https://doi.org/10.1093/biolre/ioz117

Verma, A., Verma, M., and Singh, A. (2020). Animal tissue culture principles and applications. Animal Biotechnology. 269–293. doi: 10.1016/B978-0-12-811710-1.00012-4

Vinayak, S. (2018). Influence of Epidermal Growth Factor in the In vitro Development of Bovine Preimplantation Embryos. International Journal of Pure & Applied Bioscience. 6 (5), 584-589. doi: 18782/2320-7051.6638.

Warriach, H., McGill, D., Bush, R., Wynn, P. and Chohan, K. (2015). A Review of Recent Developments in Buffalo Reproduction. Asian-Australasian Journal of Animal Sciences. 28, 451-455. http: //dx .doi. org /10.5713/ajas.14.0259

Watson, N.J., De Sousa, P., Caveney, A., Barcroft, L.C., Natale, D., Urquhart, J. and Westhusin, M.E. (2000). Impact of Bovine Oocyte Maturation Media on Oocyte Transcript Levels, Blastocyst Development, Cell Number, and Apoptosis. Biology of Reproduction. 62 (2),355–364, https :// doi.org /10.1095/biolreprod62.2.355.

Widayati, D.T. and Pangestu, M. (2020). Effect of follicle-stimulating hormone on Bligon goat oocyte maturation and embryonic development post in vitro fertilization. Veterinary world. 13 (11), 2443-2446. doi: www.doi.org /10.14202/vetworld.2020.2443-2446

Yang, R.F., Xiong, X.R. and Zi, X.D. (2019). Effect of cysteine, insulin-like growth factor-1 and epidermis growth factor during in vitro oocyte maturation and in vitro culture of yak-cattle crossbred embryos. Journal of Applied Animal Research. 47 (1), 463-466. DOI: 10.1080/09712119.2019.1663353

Yokoo, M., Kimura N., Abe, H.and Sato, E. (2008). Influence of hyaluronan accumulation during cumulus expansion on in vitro porcine oocyte maturation. Zygote. 16, 309-314. doi: 10. 1017/ S0967199408004954

Yokoo, M., Shimizu, T., Kimura, N., Tunjung, W.A.S., Matsumoto, H., Abe, H., Sasada, H., Rodríguez-Martínez, H.and Sato, E. (2007). Role of the hyaluronan receptor CD44 during porcine oocyte maturation. The Journal of reproduction and development. 53 (2), 263-270. doi: 10 .1262 /jrd. 18047.

Yousef, N., Hussein, H., Badr, M. and Ezzat, A. (2018). Effect of Epidermal Growth Factors (EGF) on the Maturation and Developmental Competence of Buffalos' Oocytes and Embryo Stages *In Vitro*. SVU-International Journal of Veterinary Sciences. 1, 85-94.

Yu, Y., Yan, J., Li, M., Yan, L., Zhao, Y., Lian, Y., li, R., Liu, P. and Qiao, J. (2012). Effects of combined epidermal growth factor, brain-derived neurotrophic factor and insulin-like growth factor-1 on human oocyte maturation and early fertilized and cloned embryo development. Human reproduction (Oxford, England). 27, 2146-2159. Doi :10 .1093 /humrep /des099

Zhao, J. Z., Zhou, W., Zhang, W., Ge, H.S., Huang, X.F. and Lin, J.J. (2009). In vitro maturation and fertilization of oocytes from unstimulated ovaries in infertile women with polycystic ovary syndrome. Fertil. Steril. 91 (6), 2568-2571. doi: 10 .1016 /j. fertnstert.2008.03.059.

تأثير بيئة الانضاج، الهرمونات الجونادوتروبين ، البروتينات ، عوامل النمو وحمض الهيالورونيك على معدل نضج بويضات الجاموس في المعمل

أحمد حمدى خضر[1]، عاصم عبدة رمضان[1]، أحمد محمد الشربيني[1] و أحمد صبري عبدون[2]

[1]قسم الانتاج الحيواني كلية الزراعية جامعة عين شمس.

[2]قسم التناسل والتلقيح الاصطناعي في الحيوان، الشعبة البيطرية، المركز القومي للبحوث

أجريت هذه الدراسة لمعرفة تأثير بيئة الانضاج، الهرمونات الجونادوتروبينه، البروتينات، عوامل النمو وحمض الهيالورونيك على معدل إنضاج بويضات الجاموس. تم سحب البويضات من الحويصلات المبيضية وتصنيفها وفقًا لمظهرها الخارجي إلى 4 درجات، وتم استخدام البويضات ذات الجودة الممتازة والجيدة. تم اختبار إنضاج البويضات باستخدام بيئة TCM199 أو CR1aa أو DMEM (التجربة 1). 10 ميكروجرام FSH/ ملي مع أو بدون 50 وحدة دولية من eCG/ملي (التجربة 2). 10% FSH، 4 ميكروجرام BSA/ملي أو 1 ميكروجرام PVA/ملي (التجربة 3). مجموعة الكنترول، 10 ميكروجرام / مل IGF-1 أو 10 ميكروجرام / مل EGF (التجربة 4) و0، 2.5، 5 و10 ميكروجرام من حمض الهيالورونيك (التجربة 5). في جميع التجارب تمت زراعة البويضات لمدة 22-24 ساعة ثم فحص النضج السيتوبلازمي والنووي. النتائج تشير إلى أن TCM199 حققت أفضل نضج سيتوبلازمي ونووي. أدت إضافة FCS إلى بيئة الإنضاج إلى تحسين معدل النضج. تدعيم بيئة الانضاج بهرمون eCG مع أو بدون FSH وكذلك عوامل النمو أدى الي تحسين نسبة الانضاج. أظهرت إضافة حمض الهيالورونيك بنسبة 2.5 ميكروجرام/1ملي لتحسين نسبة الانضاج النووي والسيتوبلازمي. والخلاصة، فإن تدعيم بيئة TCM199 بـ FCS أو eCG + FSH أو عوامل النمو أو حمض الهيالورونيك يزيد من معدل النضج السيتوبلازمي والنووي معملياً لبويضات الجاموس.