Full Length Research Paper

Effect of medium type and luteinizing hormone (LH) on *in vitro* maturation of Egyptian buffalo oocytes

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This study was carried out to evaluate the maturation of buffalo oocytes *in vitro*. In the first experiment, oocytes were cultured for 22 to 24 h in TCM-199, CR1aa and mSOF with or without luteinizing hormone (LH). Results show that all media with LH were better than media without LH; maturation rate was low in mSOF without LH and high in TCM–199 and CR1aa with LH. Moreover in comparison, TCM–199+LH with CR1aa + LH, 1282 oocytes were cultured. Maturation rate was assessed by evaluation of degree of cumulus cells expansion and meiotic development. Results indicate that recovery rate ranged between 2.4 to 2.8. Moreover, results revealed that maturation rate, telophase and metaphase II stages were higher for oocytes matured in TCM-199 + LH than oocytes matured in CR1aa + LH. In the second experiment, oocytes were matured *in vitro* in TCM 199+LH for investigating the effect of oocytes quality (excellent, good and denuded) and culture system (single or group). Excellent oocytes and group culture system were significantly higher in maturation rate and metaphase II stage. In conclusion, using TCM-199+LH, excellent oocytes and group culture system resulted in an increase of oocytes matured *in vitro*.

Key words: LH, *In vitro* maturation, Egyptian buffalo, types media.

INTRODUCTION

In most mammalian species, oocytes are formed during fetal life and are arrested at the prophase stage of the first meiotic division until around the time of ovulation (Wu et al., 1997). Numerous studies have demonstrated that intercellular communication between oocytes and granulosa cells is necessary for oocyte growth and maturation (Cecconi and Colonna, 1996). Also, Ge et al. (2008) reported that the development competence of bovine oocytes was reduced when the number of attached cumulus cells was reduced. However, conflicting results were reported by Kim et al. (1996) who

reported no difference in developmental competence, even though the cumulus cell numbers from COCs were decreased. As was reported by Shirazi et al. (2007), preantral granulosa cells produce specific factors that stimulate oocyte growth, independently of oocyte-cumulus cell contact. Granulosa/cumulus cells also play an essential role in promoting full oocyte maturation by mediating the positive effects of gonadotrophins (Moor et al., 1996). The competence of buffalo oocytes is influenced by biological factors (Nandi et al., 2000 a, b) and environmental factors (Nandi et al., 2001) such as follicle size, oocyte diameter, presence or absence of corpus luteum in the ovary and environmental tem-perature. Also, oocyte ability to resume meiosis may also be dependent on the genetic factors, as shown by (Gilchrist and Thompson, 2007).

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Expansion of cumulus cells depends largely on the culture media used for maturation of the oocytes. Different culture media such as TCM-199 (Kharche et al., 2006), minimum essential medium (MEM) (Bavister et al., 1992) and Ham's F-10 (Tamilmani et al., 2005; Arunakumari et al., 2007) have been used for *in vitro* maturation of mammalian oocytes. Supplementation of serum of any source, gonadotrophins and steroids in the culture media enhance the expansion of cumulus cells (Farag et al., 2009).

Supplementation of the IVM media with hormones, especially luteinizing hormone (LH), follicle stimulating hormone (FSH) and estradiol, alone or in combination, has been considered by some authors to be essential for obtaining high maturation rates (Jainudeen et al., 1993) whereas others have reported comparable maturation rates without the use of any hormone (Madan et al., 1994).

The choice of an individual hormone or combination of hormones depends partly on the type of basic culture medium used. Totey et al. (1993) observed that whereas addition of LH improved the maturation rate in Ham's F-10, estradiol and FSH failed to synergize with LH.

In TCM-199, LH failed to enhance the maturation rate, but the addition of FSH and esradiol did. Following the supplementation of TCM-199 with a serum source like fetal calf serum (FCS) or oestrous bovine serum (OBS), the combination of esradiol, LH and FSH was as effective as a combination of esradiol with either gonadotrophin in terms of nuclear maturation and fertilization rates. Many authors found that nuclear maturation rates was around 70 – 80% when used TCM-199, a serum such as FBS or OBS along with 5 μg / ml FSH-P (Farag et al., 2009, 2010; Tsafriri et al., 2005).

Now it's very important to improve the quality and quantity of this species. But this is severally hampered by its own low reproduction efficiency, which can be overcome by the different reproductive biotechniques like Embryo Transfer Technology, *in vitro* fertilization, embryonic stem cell production and cloning (Deshmukh et al., 2010).

The oocyte harvest per ovary can range from 0.46 (Totey et al., 1991) to 3.0 (Duran et al., 1996), an average of only 1.5 per ovary. By aspiration of 2 – 8 mm diameter follicles, the average recovery of total oocytes per ovary has been reported to vary from 0.7 (Totey et al., 1992), 1.7 (Das et al., 1996) to 2.4 (Kumar et al., 1997).

The average recovery of acceptable quality oocytes per ovary is further reduced to only 0.4 (Madan et al., 1994), 0.9 (Das et al., 1996) to 1.7 (Samad et al., 1998).

In order to increase our knowledge in the area of *in vitro* maturation of buffalo oocytes, this study was conducted to determination the best and suiTable media for *in vitro* maturation of Egyptian buffalo oocytes, and evaluates the *in vitro* maturation process on the basis of cytoplasmic maturation (expansion of cumulus cells) and nuclear maturation (nucleus staining).

MATERIALS AND METHODS

Experimental design

This study included two experiments as follows:

First experiment

This experiment aimed at testing 6 different types of media. A number of 3551 oocytes were used and divided into six groups; each group was cultured in one maturation medium. Different media composition and number of tested oocytes are presented in (Table 1). Then, 1282 oocytes were used for comparing maturation rate of oocytes cultured in M1 and M3 media (Table 1). Using 643 and 639 oocytes, maturation rate for M1 and M3 media was calculated, respectively. Degree of cumulus cells expansion and states of nucleus were used to assess maturation rate after 24 h in culture by expansion degree of cumulus cells.

Second experiment

According to the results of the first experiment, oocytes (n = 1117) were cultured in M1 medium to study the effect of oocyte quality (excellent, good and denuded), and culture system (single and grouping culture) on IVM rates of oocytes. Moreover, to compare between the effect of right and left ovary on oocyte production, 217 right and 209 left ovaries were used.

Collection of ovaries

Ovaries from sexually mature buffalo were obtained at a local slaughterhouse and were transported to the laboratory within 2 to 3 h of slaughtering. The ovaries were stored in insulated thermos flask containing normal saline (0.9% NaCl) supplemented with 50 μg gentamycin /ml at 25 to 30°C.

Oocytes collection and classification

At the laboratory, cumulus oocyte complexes (COCs) were obtained by aspiration of follicles (2 to 8 mm in diameter) using a 10 ml sterile syringe and an 18 G disposable needle. The aspirated COCs from follicles were divided into four categories according to homogenous of cytoplasm and number of cumulus cells layers (Blondin and Sirard, 1995).

In vitro maturation

Selected oocytes were cultured in different media as shown in (Table 1) for 24 h at 38°C, 5% CO_2, and more than 95% humidity.

Evaluation of maturation

Oocyte maturation process was evaluated by two criteria:

Expansion of cumulus cells

The criteria used for assessing the degree of cumulus cells

Table 1. Composition of different types of media and number of tested oocytes.

Medium code	Composition	Number of tested oocyte
M1	TCM-199 + 10 % FCS + 0.02 U FSH / ml + 0.023 U LH / ml + 50 μg Gentamycin sulfate / ml + 1 μg Estradiol 17 β / ml.	574
M2	TCM-199 + 10 % FCS + 0.02 U FSH / ml + 50 μg Gentamycin sulfate / ml + 1 μg Estradiol 17 β / ml.	603
M3	CR1aa + 10 % FCS + 0.02 U FSH / ml + 0.023 U LH / ml + 50 μg Gentamycin sulfate / ml + 1 μg Estradiol 17 β / ml.	598
M4	CR1aa + 10 % FCS + 0.02 U FSH / ml + 50 μg Gentamycin sulfate / ml + 1 μg Estradiol 17 β / ml.	593
M5	m-SOF + 10 % FCS + 0.02 U FSH / ml + 0.023 U LH / ml + 50 μg Gentamycin sulfate / ml + 1 μg Estradiol 17 β / ml.	597
M6	m-SOF + 10 % FCS + 0.02 U FSH / ml + 50 μg Gentamycin sulfate / ml + 1 μg Estradiol 17 β / ml.	586

expansion after 24 h of IVM described by Kobayashi et al. (1994) is as follows:

Degree 0: Slight or no expansion

Cumulus cells were adherent to the zona pellucida.

Degree 1: Moderate cumulus cells expansion

Approximately 70% of cumulus cells were homogeneously spread.

Degree 2: Full cumulus cells expansion

All cumulus cells were homogeneously spread.

Staining of the nucleus

Upon completion of maturation time, the cumulus cells are removed from the matured oocytes. The denuded oocytes were washed with DPBS and stained by a rapid staining method (Khalil, 2003). Briefly, matured oocytes were fixed overnight in glacial acetic acid: ethanol absolute (1: 3). Oocytes then were stained with 1% aceto–orcein and examined for nuclear morphology using inverted microscope. The stages of nuclear maturation were assessed as germinal vesicle (GV), germinal vesicle break down (GVBD), metaphase I (MI), anaphase I and telophase I (AITI) or metaphase II (MII). Oocytes that reached metaphase II stage were considered as matured according to Mtango et al. (2003).

Statistical analysis

Data of the effect different media on *in vitro* maturation of buffalo oocyte complexes were analyzed using one way analysis of variance (ANOVA) while, analysis of data for the effect of oocyte quality, and culture system was performed by using factorial design method. Differences were considered significant at the 0.05 level by using Duncan's Multiple Range Test procedure (Duncan, 1955).

RESULTS AND DISCUSSION

Oocytes recovery and quality

Although, abattoir derived ovaries provide a cheap and abundant source of oocytes, a serious problem associated with the production of buffalo embryos through IVMFC is the very poor recovery of total and good quality immature oocytes from slaughterhouse ovaries. The results reveal that there was a significant difference between the right and left ovaries for oocyte quality produced and recovery rate as shown in Table 2.

The recovery rate of oocyte in this study is lower than that recorded by Khan et al. (1997) (5.3 to 6.0) and Ganguli et al. (1998) (8.0); also, Kadoom (1995) (3.33) in buffalo and higher than that reported by Datta (1994) (1.11) in buffalo; Das et al. (1996) (1.7) and Datta and Goswami (1998) (0.96) in buffalo.

The results obtained in the present study are in agreement with those of Kumar et al. (1997), Abd-Eltawab (2003) in buffaloes. Regarding the types of oocyte quality, results obtained here are nearly in accordance with those reported by Ganguli et al. (1998) and Abd-Eltawab (2003) and disagree with those reported by Mahmoud (2001).

Low oocyte yield in buffalo may be due to: (1) a considerably lower number of primordial follicle reserve (12000 in Surti buffalo (Danell, 1987) to 19000 in Nilli-Ravi buffalo (Samad and Nasseri, 1979), compared with 150000 in cattle (Erickson, 1966); (2) the low number of antral follicles at all stages of the estrous cycle (Kumar et al., 1997; Manik et al., 1998); (3) a high incidence of deep atresia, reported to be 82% (Ocampo et al., 1994) to 92%

Table 2. Comparison between oocytes production from the right and left ovary in Egyptian buffaloes.

Trait		Right ovary	Left ovary
Total oocyte		585	532
Excellent	Total (%)	162 (27.7)	106 (19.9)
	Mean $\pm$ S.E	28.3 $\pm$ 1.1[a]	20.2 $\pm$ 1.3[b]
Good	Total (%)	335 (57.3)	334 (62.8)
	Mean $\pm$ S.E	57.2 $\pm$ 1.1[b]	62.4 $\pm$ 1.7[a]
Denuded	Total	89 (15.2)	93 (17.5)
	Mean $\pm$ S.E	15.3 $\pm$ 0.7[b]	17.6 $\pm$ 0.9[a]
Recovery rate	Mean $\pm$ S.E	2.7 $\pm$ 0.04[a]	2.5 $\pm$ 0.03[b]

Means with different superscript (a, b) within the same raw are significantly different (P ≤0.05).

(Palta et al., 1998) in ovarian follicles from slaughter-house ovaries; slaughtering of buffaloes in a sub-fertile, unproductive state, with aged and detrimental body condition (Nandi et al., 2002).

The variations in the oocyte recovery of follicular oocytes may be attributed to the difference in age, health, nutritional and genetic status of the female buffalo donors (Mahmoud, 2001).

Effect of oocytes quality on IVM of Egyptian buffalo oocytes

In the present study, data shown in Table 3 showed that excellent quality buffalo oocytes surrounded by multi-layers of cumulus cells with a homogenous cytoplasm had a significantly higher maturation rate compared with oocytes of good quality (Figure 1). Although, mean of expansion was greater for good quality oocytes than excellent quality oocytes (15.2 $\pm$ 2.1 vs. 7.5 $\pm$ 0.6, respectively) as shown in Figure 2, the mean of maturation rate was higher in excellent quality oocytes than good quality ones (90.6 $\pm$ 2.2 vs. 71.9 $\pm$ 2.2, respectively).

Analysis of data revealed that means of telophase and anaphase and metaphase II stage were higher in excellent oocytes (1.7 $\pm$ 0.2 and 2.7 $\pm$ 0.3, respectively) than good and denuded oocytes (0.9 $\pm$ 0.2 and 1.5 $\pm$ 0.2 and 0.4 $\pm$ 0.1 and 0.3 $\pm$ 0.1, respectively). Moreover, regarding the stages of nucleus state of oocytes, results showed that good quality oocytes had a higher means for GV, GVBD and MI (10.0 $\pm$ 1.6, 1.9 $\pm$ 0.2 and 0.9 $\pm$ 0.2, respectively) than excellent (2.1 $\pm$ 0.3, 0.6 $\pm$ 0.1 and 0.4 $\pm$ 0.1, receptively) and denuded quality oocytes (2.5 $\pm$ 0.2, 1.3 $\pm$ 0.2 and 0.6 $\pm$ 0.1, receptively) as shown in Figure 3.

An important indication of the attainment of both nuclear and cytoplasmic maturation of oocytes is thus the layers of cumulus cells surrounding the oocytes. Hence, the cumulus expansion can importantly be used to assess the *in vitro* maturation rate of oocytes (Gupta et al., 2005; Kidson, 2005).

Oocytes are selected for embryo production on the basis of compaction of cumulus-corona investment and the homogeneity of ooplasm (Katska-Ksiazkiewicz et al., 2007). On the other view, oocytes with expanded, clumped cumulus cells complex and irregular cytoplasm exhibited decreased *in vitro* maturation (De Loos et al., 1989). Schoevers et al. (2007) explained the important role of cumulus cells in IVM. Since, IVM provide energy source which necessary for oocyte maturation or may be provide other factors or hormones capable of regulating maturation. Also, the cumulus cells support the penetrability of the oocyte by the sperm by preventing the zona from hardening, caused by the premature exocytosis of the cortical granules (Schoevers et al., 2007).

Failure of Egyptian buffalo oocytes to develop might be attributed to unsuitable hormonal profile, poor quality of the oocytes due to old age of animals, and mainly due to long anestrous. Moreover, buffalo oocytes surrounded by multi-layers of cumulus cells had a significantly higher IVM rates than partially surrounded or denuded oocytes (Abdoon et al., 2001).

Effect of type of culture media and LH supplementation on IVM of Egyptian buffalo oocytes

The effect of maturation media types and addition of LH on *in vitro* maturation of buffalo oocytes is shown in Tables 4 and 5. The percentage and mean of expansion oocytes and maturation rate were higher in TCM-199 medium than CR1aa and m-SOF media. Moreover, the maturation rate was greater in medium with LH vs. without (TCM-199; 95.68 $\pm$ 0.40 vs. 77.61 $\pm$ 0.90, CR1aa; 91.74 $\pm$ 0.75 vs. 74.71 $\pm$ vs. 0.42, and m-SOF; 57.17 $\pm$

Table 3. Effect of oocyte quality on *in vitro* maturation of Egyptian buffalo oocytes.

Trait		Excellent	Good	Denuded
Total oocyte		273	669	187
Expanded	Total %	254 (93.0)	516 (77.1)	0
	Mean $\pm$ S.E	7.5 ± 0.6^b	15.2 ± 2.1^a	0.0^c
Non-expanded	Total	19 (7)	153 (22.9)	0
	Mean $\pm$ S.E	0.6 ± 0.1^b	4.5 ± 0.5^a	0.0^c
Maturation rate	Min. %	60	40	0
	Max. %	100	84.4	0
	Mean $\pm$ S.E	90.6 ± 2.2^a	71.9 ± 2.2^b	0.0^c
Germinal vesicle	Total %	72 (28.3)	339 (65.7)	86 (46.0)
	Mean $\pm$ S.E	2.1 ± 0.3^b	10.0 ± 1.6^a	2.5 ± 0.2^b
Germina vesicle break down	Total %	20 (7.9)	64 (12.4)	46 (25.0)
	Mean $\pm$ S.E	0.6 ± 0.1^c	1.9 ± 0.2^a	1.3 ± 0.2^b
Metaphase I	Total	14 (5.5)	32 (6.2)	22 (11.8)
	Mean $\pm$ S.E	0.4 ± 0.1^b	0.9 ± 0.2^a	0.6 ± 0.1^{ab}
Telophase& Anaphase	Total %	59 (23.2)	31 (6.0)	12 (6.4)
	Mean $\pm$ S.E	1.7 ± 0.2^a	0.9 ± 0.2^b	0.4 ± 0.1^c
Metaphase II	Total %	93 (36.6)	52 (10.1)	11 (5.9)
	Mean $\pm$ S.E	2.7 ± 0.3^a	1.5 ± 0.2^b	0.3 ± 0.1^c

Means with different superscript (a, b, c) within the same row are significantly different at (P ≤0.05).
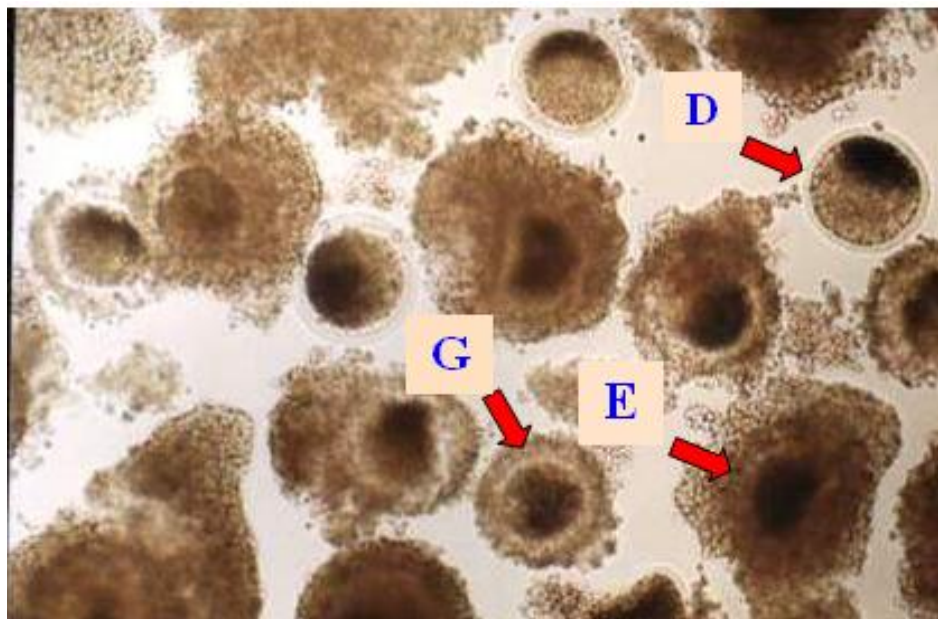
Figure 1. Quality of Egyptian buffalo oocytes. E = Excellent oocyte, G = good oocyte, D = denuded oocyte.
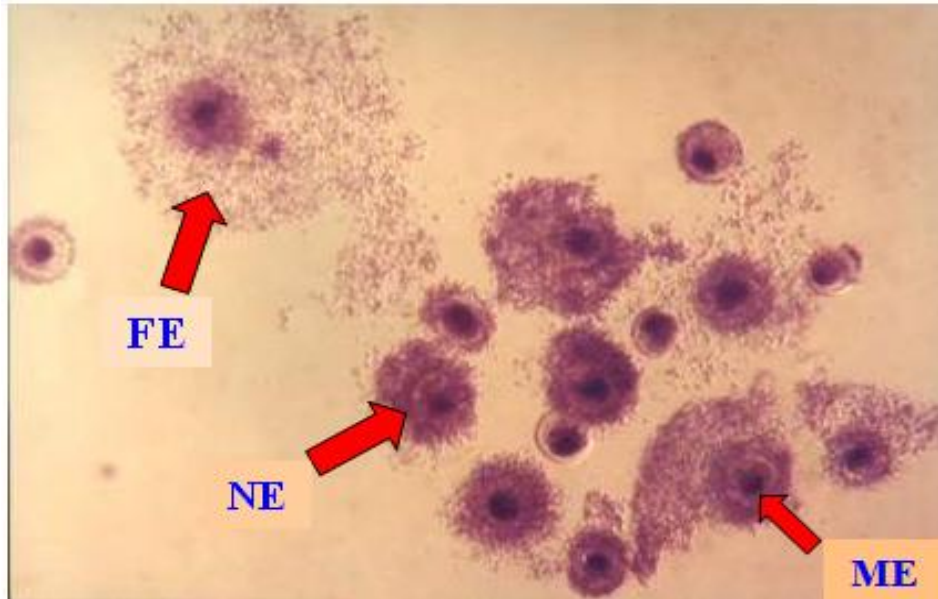
Figure 2. Expansion degree of cumulus cells around the bufflo oocytes. NE = No expansion, ME = moderate expansion, FE = full expansion.

1.06 vs. 40.92 ± 0.68, respectively).

Also, Table 5 reveals that TCM-199 + LH are the best medium for IVM of buffalo oocytes when compared with CR1aa +LH since, the maturation rate was 94.8 ± 0.48 vs. 91.7 ± 0.39, respectively. The best maturation medium (TCM-199 + LH) had a higher mean of telophase and anaphase and M II (8.8 ± 0.36 and 16.4 ± 0.45, respectively) while, CR1aa + LH medium had lower means (7.2 ± 0.33 and 14.1 ± 0.35, respectively).

Many factors affect maturation of *in vitro* cultured oocytes among which are types of maturation medium, and media supplementation. Expansion of cumulus cells depends largely on the culture media used for maturation of the oocytes (Camargo et al., 2006; Cox and Alfaro, 2007; Wan et al., 2009) and presence of gonadotropin inmaturation media (Jamil et al., 2007).

To induce full maturation of the nucleus and cytoplasm in bovine follicular oocytes, several important factors, such as addition of FSH, LH, estradiol, granulosa cells and serum to the culture media is necessary (Wang et al., 2007).

The maturation rate ranged between 40.92 to 95.68% as shown in Table 5. In some reports, however, maturation rate ranged between 45.00 to 74.19% (Ganguli et al., 1998). Abbas (1998) who revealed that supplementation of the IVM media with hormones, especially LH, FSH and oestradiol, alone or in combination, resulted in high maturation rates whereas, other have reported comparable maturation rates without the use of any hormone (Madan et al., 1994).

Results of the present study showed that the maturation rate reached the maximum on addition of LH to the maturation media. These results are in agreement with (Chung et al., 1990) who revealed that LH has been shown to have a positive effect on maturation. However, the effect of the gonadotropins and their relative importance on *in vitro* maturation, subsequent fertilization and early development is still controversial according to Goto and Iritani (1992). Although, FSH and LH are by no means necessary for spontaneous oocyte maturation, it is generally believed that these hormones improve oocyte cytoplasmic maturation by significantly altering a range of cumulus cell activities. It is unclear, however, if this beneficial effect of gonadotrophins is mediated by changes in cumulus cell metabolic activity (Sutton et al., 2003; Cecconi et al., 2008).

One mechanism by which LH may enhance IVM of bovine oocytes is through modifying the nutritional environment to increase the energy available for the oocyte to support its functional role in fertilization and subsequent development (Harper and Brackett, 1993). Moreover, Zuelke and Brackett (1992) revealed that the net metabolism effect of LH exposure during IVM could be shown as increased glycolysis combined with increased mitochondrial glucose oxidations; this profile implicated LH in effecting increased tricarboxylic acid (TCA) cycle activity within cumulus cell-enclosed oocytes

Effect of culture system on IVM of Egyptian buffalo oocytes

The two IVM systems (group or single) showed significant differences in all traits. The mean of expansion,
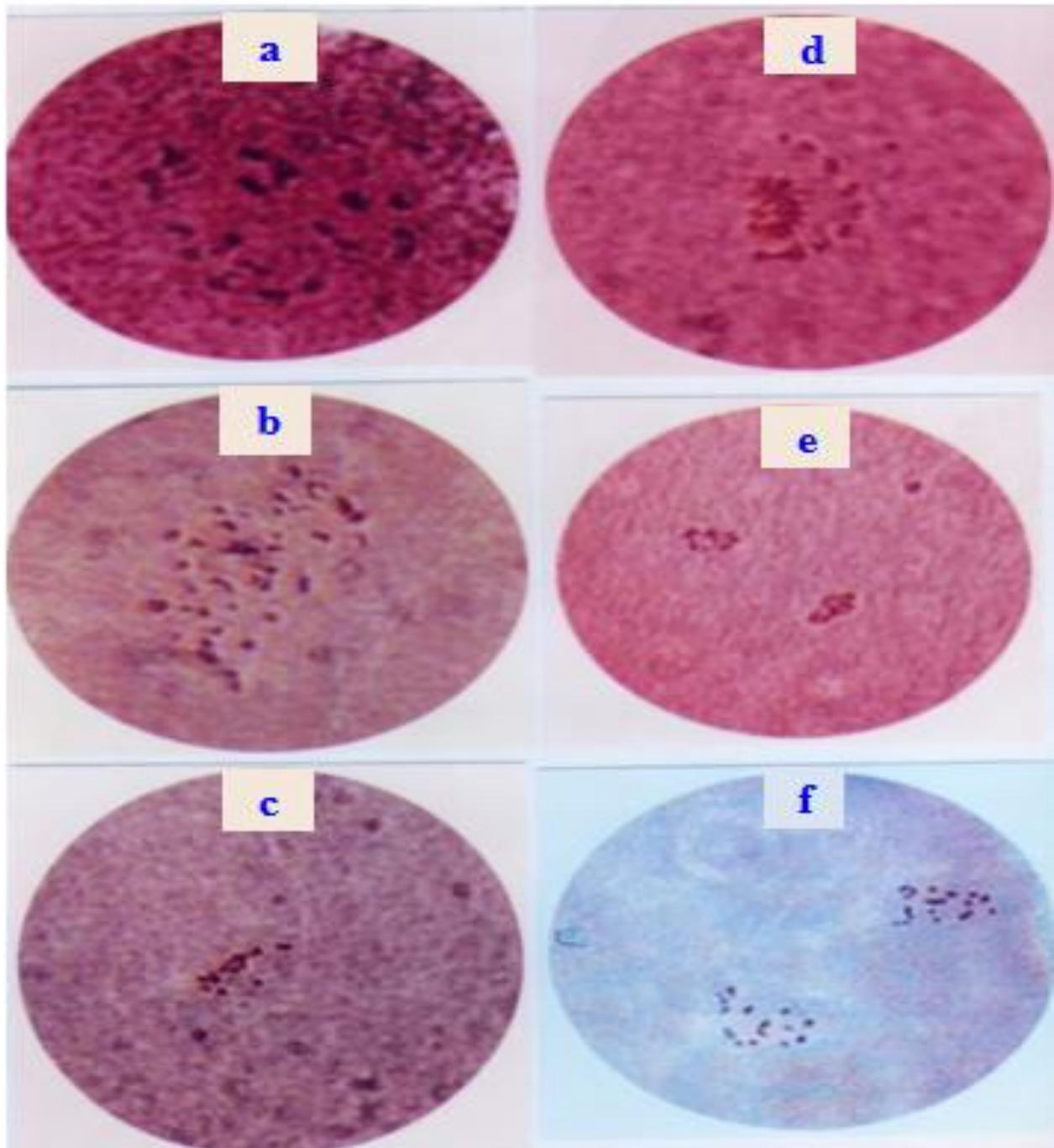
Figure 3. Different stages of nuclear maturation of Egyptian buffalo oocytes. a: Germinal vesicle (GV), b: germinal vesicle break down (GVBD), c: metaphase I (M I), d: anaphase I (Anaph I), e: telophase I (Teloph. I), f: metaphase II (M II).

maturation rate and Metaphase II of oocytes was higher in grouping culture than single culture (12.6 ±1.6 vs. 2.5 ± 0.3, 59.0 ± 6.0 vs. 9.1 ± 5.3 and 2.2 ± 0.2 vs. 0.8 ± 0.1), respectively (Table 6). On the contrary, concerning the effect of culture system and oocyte quality on IVM of buffalo oocytes, the statistical analysis of data in Table 7 showed that there was no significant differences between

two culture systems in case of denuded oocytes. While excellent and good oocytes showed a significant difference between the single and grouping culture system.

Moreover, Table 7 shows that excellent and good oocytes had a significant higher means in grouping culture system than in single culture system concerning

Table 4. Effect of media types on *in vitro* maturation of Egyptian buffalo oocytes.

Media type	Total oocyte	Expanded Total (%)	Expanded Mean ± S.E	Non-expanded Total (%)	Non-expanded Mean ± S.E	Maturation rate % Mean ± S.E
TCM-199 + LH	574	547 (95.3)	54.7 ± 0.96[a]	27 (4.7)	2.7 ± 0.21[e]	95.68 ± 0.40[a]
TCM-199	603	467 (77.4)	46.7 ± 0.93[b]	136 (22.6)	13.6 ± 0.86[c]	77.61 ± 0.90[c]
CR1aa + LH	598	548 (91.6)	54.8 ± 0.83[a]	50 (8.4)	5.0 ± 0.52[d]	91.74 ± 0.75[b]
CR1aa	593	443 (74.7)	44.3 ± 0.86[b]	150 (25.3)	15.0 ± 0.39[c]	74.71 ± 0.42[d]
m-SOF + LH	597	341 (57.1)	34.1 ± 0.87[c]	256 (42.9)	25.6 ± 0.99[b]	57.17 ± 1.06[e]
m-SOF	586	240 (41.0)	24.0 ± 0.83[d]	346 (59.0)	34.6 ± 0.96[a]	40.92 ± 0.68[f]

Means with different superscript (a, b, c, d, e, f) within the same column are significantly different (P ≤ 0.05).

Table 5. Comparison between effects of two different media supplemented with LH on *in vitro* maturation of Egyptian buffalo oocytes

Trait		TCM – 199 + LH	CR1aa + LH
Total oocyte		643	639
Expanded	Total (%)	609 (94.7)	586 (91.7)
	Mean ± S.E	60.9 ± 1.09[a]	58.6 ± 0.79[a]
Non-expanded	Total (%)	34 (5.3)	53 (8.3)
	Mean ± S.E	3.4 ± 0.37[a]	5.3 ± 0.30[a]
Maturation rate	Mean ± S.E	94.8 ± 0.48[a]	91.7 ± 0.39[b]
GV	Total (%)	230 (37.8)	251 (42.8)
	Mean ± S.E	23.0 ± 0.49[b]	25.1 ± 0.67[a]
GVBD	Total (%)	97 (15.9)	91 (15.5)
	Mean ± S.E	9.7 ± 0.45[a]	9.1 ± 0.35[a]
MI	Total (%)	30 (4.9)	29 (4.9)
	Mean ± S.E	3.0 ± 0.30[a]	3.1 ± 0.23[a]
TI	Total (%)	88 (14.4)	72 (12.3)
	Mean ± S.E	8.8 ± 0.36[a]	7.2 ± 0.33[b]
MII	Total (%)	164 (26.9)	141 (24.1)
	Mean ± S.E	16.4 ± 0.45[a]	14.1 ± 0.35[b]

Means with different superscript (a, b) between the two treatments are significantly different (P ≤0.05). GV, Germinal vesicle; GVBD, germinal vesicle break down; MI, metaphase I;   TI, Telophasei; MII, metaphase II.

maturation rate and metaphase II (97.8 ± 1.1 vs. 83.5 ± 3.5 and 79.2 ± 0.9 vs. 64.7 ± 3.7 and 4.1 ± 0.2 vs. 1.4 ± 0.2 and 2.2 ± 0.2 vs. 0.9 ± 0.2), respectively. In addition, in the case of grouping culture the excellent oocytes had a significant higher means than good oocytes for maturation rate, telophase and anaphase and metaphase II. While under single culture system the excellent oocytes had a significant higher means in the case of

maturation rate and telophase and anaphase only.

Results of the current research are in agreement with those of many authors who revealed that cumulus oocyte complexes aspirated from antral follicles of slaughtered animals are usually matured, fertilized and cultured in groups (Hangemann et al., 1999).

On the other hand, results reported here disagreed with those found by Fukui et al. (2000) who indicated that a

Table 6. Comparison between the two types of culture system on *in vitro* maturation of Egyptian buffalo oocytes.

Trait		Single culture	Grouping culture
Total oocyte		255	874
Expanded	Total %	126 (49.4)	644 (73.7)
	Mean ± S.E	2.5 ± 0.3[b]	12.6 ± 1.6[a]
Non-expanded	Total %	44 (17.3)	128 (14.6)
	Mean ± S.E	0.9 ± 0.1[b]	2.5 ± 0.5[a]
Maturation rate	Mean ± S.E	9.1 ± 5.3[b]	59.0 ± 6.0[a]
Germinal vesicle	Total %	73 (57.9)	424 (65.8)
	Mean ± S.E	1.4 ± 0.2[b]	8.3 ± 1.1[a]
Germinal vesicle break down	Total %	45 (35.7)	84 (13.0)
	Mean ± S.E	0.9 ± 0.1[b]	1.6 ± 0.2[a]
Metaphase I	Total %	21 (16.7)	47 (7.3)
	Mean ± S.E	0.4 ± 0.1[b]	0.9 ± 0.1[a]
Telophase & Anaphase	Total %	28 (22.2)	74 (11.5)
	Mean ± S.E	0.5 ± 0.1[b]	1.5 ± 0.2[a]
Metaphase II	Total %	42 (33.3)	114 (17.7)
	Mean ± S.E	0.8 ± 0.1[b]	2.2 ± 0.2[a]

Means with different letters (a, b) within the same row are significantly different at (P ≤0.05).

Table 7. Effects of interaction between quality of oocytes and type of culture on *in vitro* maturation of Egyptian buffalo oocytes.

Trait		Single culture			Grouping culture		
		Excellent	**Good**	**Denuded**	**Excellent**	**Good**	**Denuded**
Total oocyte		85	85	85	188	584	102
Expanded	Total (%)	71 (83.5)	55 (64.7)	0.0	183 (97.3)	461 (78.9)	0.0
	Mean ± S.E	4.2 ± 0.2[c]	3.2 ± 1.8[c]	0.0 ± 0.0[d]	10.8 ± 0.5[b]	27.1 ± 7.2[a]	0.0 ± 0.0[d]
Non-expanded	Total (%)	14 (16.5)	30 (35.3)	0.0	5 (2.7)	123 (21.1)	0.0
	Mean ± S.E	0.8 ± 0.2[c]	1.8 ± 0.2[b]	0.0 ± 0.0[d]	0.3 ± 0.1[cd]	7.2 ± 0.4[a]	0.0 ± 0.0[d]
Maturation rate	Mean ± S.E	83.5 ± 3.5[b]	64.7 ± 3.7[c]	0.0 ± 0.0[d]	97.8 ± 1.1[a]	79.2 ± 0.9[b]	0.0 ± 0.0[d]
Germinal vesicle	Total (%)	14 (19.7)	18 (32.7)	41 (48.2)	58 (31.7)	321 (69.6)	45 (44.1)
	Mean ± S.E	0.8 ± 0.2[c]	1.1 ± 0.2[c]	2.4 ± 0.2[b]	3.4 ± 0.1[b]	18.9 ± 0.8[a]	2.6 ± 0.2[b]
Germinal vesicle break down	Total (%)	10 (14.1)	14 (25.5)	21	10 (5.5)	50 (10.8)	24 (23.5)
	Mean ± S.E	0.6 ± 0.2[c]	0.8 ± 0.2[bc]	1.2 ± 0.3[b]	0.6 ± 0.2[c]	2.9 ± 0.2[a]	1.4 ± 0.2[b]
Metaphase I	Total (%)	7 (9.9)	3 (5.5)	11	7 (3.8)	29 (6.3)	11 (10.8)
	Mean ± S.E	0.4 ± 0.2[b]	0.2 ± 0.1[b]	0.6 ± 0.2[b]	0.3 ± 0.1[b]	1.7 ± 0.2[b]	0.6 ± 0.2[b]

Table 7. Contd.

Telophase & Anaphase	Total (%)	19 (2.7)	5 (9.1)	4	40 (21.9)	26 (5.6)	8 (7.8)
	Mean ± S.E	1.1 ± 0.1^b	0.3 ± 0.1^c	0.2 ± 0.1^c	2.4 ± 0.3^a	1.5 ± 0.2^b	0.5 ± 0.2^c
Metaphase II	Total (%)	23 (32.4)	15 (27.3)	4	70 (38.3)	37 (8.0)	7 (6.9)
	Mean ± S.E	1.4 ± 0.2^c	0.9 ± 0.2^{cd}	0.2 ± 0.1^e	4.1 ± 0.2^a	2.2 ± 0.2^b	0.4 ± 0.1^{de}

Means with different superscript (a, b, c, d, e) between the two culture systems and different quality of oocytes are significantly different at ($P \le 0.05$).

single oocyte culture system throughout the *in vitro* production of bovine embryos provide similar maturity, fertilizability and developmental capacity to oocytes cultured in groups.

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