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EFFECT OF MATURATION MEDIA WITH HORMONAL SUPPLEMENT ON *IN VITRO* MATURATION OF BUFFALO OOCYTES WITH DIFFERENT QUALITIES

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SUMMARY

This study aimed to evaluate the effect of maturation medium (TCM-199 and DMEM) supplemented with FSH or FSH+hCG on in vitro maturation of buffalo oocytes with different qualities. Buffaloes' ovaries were collected from slaughterhouse and oocytes were recovered by aspiration method. Oocytes quality was ranked based on the number of cumulus cell layers into excellent, good, fair and poor oocytes. Oocytes were in vitro matured in TCM-199 or DMEM media containing hormones. Both maturation media were supplemented with FSH (10 μg/ml) or HCG (10 IU/ml). Cytoplasmic or nuclear maturation was measured.

Results show significant (P<0.05) effect of medium type on cytoplasmic maturation only in fair oocytes. The effect of medium type on nuclear maturation was significant only on fair and poor oocytes, being higher with DMEM than TCM-199 (51.5 vs. 26.8% and 32.8 vs. 16.1%). The differences in nuclear maturation of excellent and good oocytes between TCM and DMEM were not significant. Excellent and good oocytes showed higher (P<0.05) maturation rate (76.7 and 70.9%) than fair and poor oocytes (40.2 and 24.6%), respectively. In general, the highest maturation rate was obtained from excellent oocytes matured by DMEM (81.3%). The highest percentage of oocytes showing full expansion was for excellent, good and fair by DMEM supplemented with FSH (79.3, 67.6 and 50%, respectively. These findings may suggest the beneficial effects of hormonal addition on cumulus cells expansion for all oocyte qualities, except poor oocytes. Maturation rate was the highest (P<0.05) for excellent, good and fair oocytes matured by DMEM supplemented with FSH plus hCG. However, the poor oocytes showed the highest (P<0.05) maturation rate with DMEM supplemented with FSH alone.

Maturation medium (DMEM) supplemented with FSH plus hCG showed the highest in vitro nuclear maturation of buffalo oocytes with different qualities, in particular excellent, good and fair oocytes. Further studies are needed for evaluation the effect of hormonal addition with FSH and/or hCG to DMEM medium on in vitro fertilization and embryo development of buffalo oocytes.

Keywords: Type of Maturation Media- Hormonal supplement- In Vitro Maturation- Buffalo Oocytes

INTRODUCTION

In Egypt, buffaloes are considered as the main dairy animals and known to suffer from many reproductive problems (El-Moghazy, 2003). Early efforts have been initiated to augment the reproductive potential of these animals using biotechnology (Madan *et al*., 1994). *In vitro* fertilization (IVF) technology provides an opportunity to produce embryos for genetic manipulation and embryo transfer (Nandi *et al*., 2002). Producing embryos by IVF is based on three subsequent techniques: oocyte *in vitro* maturation (IVM), IVF and *in vitro* culture (IVC) for cleavage up to blastocyst stage (Goswami *et al*., 2004).

Oocyte maturation is the foundation of embryo production (Abdoon, 2002). Oocyte maturation is the most critical step towards successful *in vitro* embryo production. The culture medium and selection of protein supplements and hormones for IVM play an important role in the subsequent maturation rate, and embryonic development following IVF (Bavister *et al*., 1992). Several factors such as addition of FSH, LH and their combination to culture media had been considered for maximizing success (Saeki *et al*., 1990). Little information is available on *in vitro* maturation and fertilization of buffalo oocytes (Shamiah, 2004), which is relatively poor when compared to cattle as reported by Bacci *et al*. (1991) and Totey *et al*. (1992).

The present work aimed at evaluating the effect of type of maturation media (TCM-199 and DMEM) with hormonal addition (FSH or FSH + hCG) on *in vitro* maturation of buffalo oocytes with particular reference to qualities.

MATERIALS AND METHODS

The present study was carried out in the Department of Animal Production, Faculty of Agriculture, Tanta University and Department of Animal Reproduction and Artificial Insemination, Veterinary Research Division, National Research Center, during the period from January 2010 to May 2011.

All chemicals and media used in this study were purchased from Sigma-Aldrich (Sant. Louis, MO, USA) unless otherwise mentioned.

Ovaries collection:

Ovaries were collected within 20-30 min post-slaughtering of buffalo cows from El-Moneib slaughterhouse, Giza City and transported to the laboratory within two to three h in an insulated thermos containing normal sterile saline solution (NSS, 0.9% NaCl) supplemented with 100 I.U/ml penicillin and 100 µg/ml streptomycin.. Upon arrival, ovaries were washed onetime with ethayl alcohol to minimize the risk of contamination followed by several times with warm NSS at 37°C until obtaining clear saline free from blood and then kept in water bath at 37°C for oocyte collection.

Oocyte recovery:

HEPES buffered tissue culture medium (HTCM-199, catalogue number M-2520) supplemented with four mg/ml bovine serum albumin (BSA) and 50 µg/ml Gentamycin was used as aspiration medium. Oocytes were recovered by aspiration method using 18-gauge needle attached to 10 ml disposable syringe containing one ml aspiration medium. The contents of the syringe were placed into 15 ml sterile falcon tube (Falcon, USA) and kept in water bath at 37ºC for 15 min, to allow oocytes to settle down. After settling the sediment at the bottom of the Falcon tube was aspirated using Pasture pipette and placed into in six cm diameter polysterene sterile Petri dish containing five ml aspiration medium for searching oocytes under stereo-microscope.

Assessment of oocytes quality was determined depending upon the number of cumulus cell layers surrounding the oocyte and the homogeneity of ooplasm under stereomicroscope (Nakamura, Japan) at x 32 according to Furnus *et al*. (1997) into excellent, those surrounded by compact and cumulus cell layers (≥ four layers) and homogenous evenly granular ooplasm; good, those surrounded by two to three layers of cumulus cells and homogenous evenly granular ooplasm; fair, those surrounded by one layer of cumulus cells, and poor (Denuded) those were completely devoid of cumulus cells around them and uneven ooplasm.

In vitro maturation:

Oocytes were washed two times by HEPES buffer TCM-199 (TCM199, Cat. 2415) or DEMM (Dulbecco's modified eagle medium) containing 10 IU/ml FSH, four % BSA and 50 µg/ml Gentamycin, then washed again by maturation medium (pH 7.2-7.4) and finally each class of oocytes was cultured in a separate well of a four-well culture plate (Nunclon, Denmark) containing maturation medium for 24 h at 38ºC under 5% CO_2 in CO_2 incubator. All media used in the present work were filtered using 0.2 µm (Millipore, USA) syringe filter and incubated for at least two h in a humidified atmosphere (95%) under 5% CO_2 at 38°C before culturing of the oocytes

To study the effect of hormonal addition to both maturation media, FSH (10 µg/ml) *vs.* TCM 199 + FSH (10 µg/ml) and HCG (10 IU/ml) were added to TCM-199 or DMEM media. Both types of maturation media were supplemented also with 10% FCS and 50 µg/ml Gentamycin.

Cytoplasmic maturation was measured by assessing the degree of cumulus-cells expansion as described by Abd El-Kader (2005) under stereomicroscope. Oocytes without expansion (Grade 0, Go), few expansions of cumulus layers (Grade 1, G1), moderate expansions of cumulus layers (Grade 2, G2) and full expansion of cumulus layers (Grade 3, G3) were determined. Oocytes were removed from the maturation medium and the cumulus- cells were removed by gentile repeated pipetting using 100µl pipette and then fixed in acetic acid and ethanol (1: 3) (Totey *et al*., 1993) for minimum 48 h for detection of nuclear maturation.

Two to three fixed oocytes in a drop were aspirated by an automatic pipette and placed on glass slide. A cover slip, with inert paraffin wax spots at each of its four corners, was placed directly over the center of the drop containing the oocytes. Thereafter, the oocytes were observed under stereomicroscope, the cover slip was pressed down on the oocytes until it was held firmly in place. The slide was stained with aceto orcein stain (one gm orcein+ 45 ml glacial acetic acid + 55 ml DDW) for five min and washed by aceto-glycerol (3:1).

Nuclear maturation of oocytes was carried out according to Sirard et al. (1989) into Germinal vesicle (GV), interphase chromosomes enclosed within a nuclear membrane; Germinal vesicle breakdown (GVBD), an absence of a visible nuclear membrane and the chromatin condensation was characterized by a cluster of DNA material without individual chromosomes; Anaphase I (A I), separation of homologous pairs of chromosomes and the chromosomes were pulling apart from each other and moving to the opposite poles of the spindle; Telophase 1 (T I), the two groups of equally spread homologous chromosomes reached the opposite poles of the spindle; Metaphase II, separation of homologous chromosomes with extrusion of first polar body, and Degenerated oocytes, being vacuolated or having scattered or highly condensed chromatin.

Oocytes were classified as immature oocytes, at either germinal vesicle stage (GV) or germinal vesicles break down stage (GVBD); mature oocytes, anaphase (An),

Telophase (T) and Metaphase II (M II with polar body) stages and unidentified or degenerated oocytes (Abd El-Kader, 2005).

Statistical analysis:

Data were statistically analyzed using Chi-square.

RESULTS AND DISCUSSION

Effect of type of maturation medium on IVM: Cytoplasmic maturation with TCM-199 vs. DMEM

Effect of medium type on cytoplasmic maturation was observed only on fair oocytes. However, the differences in cumulus cells expansion among excellent and good oocytes were not significant with TCM or DMEM. Yet poor oocytes failed to show full expansion with both media. Percentage of fair oocytes with full cumulus cells expansion was higher ($P<0.05$) with DMEM than TCM (Table 1).

The culture medium is one of the most important factors in maturation of follicular oocytes *in vitro* (Van De Sandt *et al*., 1990 and Palta and Chauhan, 1998) and expansion of cumulus cells depends largely on the culture media used for maturation of the oocytes (Nandi *et al*., 2002). TCM-199 is capable of supporting the *in vitro* maturation of bovine (Lonergan *et al*., 1994) and buffalo (Hammam *et al*., 1997) oocytes.

In the present study, DMEM medium showed better effect on expansion of cumulus cells than TCM-199 on IVM of fair oocytes. In the same concern, Gliedt *et al*. (1996) found that cumulus cells expansion was greater ($P<0.0001$) for bovine COC that matured in RPMI-1640 than for those that matured in TCM-199. Several authors found that cumulus cells expansion of buffalo oocytes was ($P<0.01$) for MSOF than TCM-199 medium (Ali, 2004; Barakat, 2005 and Abdel-Razik, 2007).

Comparing oocyte quality regardless type of medium, excellent oocytes showed the best ($P<0.05$) cytoplasmic maturation (55.1%) as compared to good (43.8%) or fair (20.6%) oocytes. It is of interest to note that the highest percentages of oocytes with full cumulus cell expansion (G3) in relation to medium type and oocyte quality was obtained with excellent buffalo oocytes matured by TCM-199 (56.5%), which was associated with revisable percentages of oocytes without expansion (G0, Table 1).

In accordance with the present results, Abdel-Razik (2007) found that the percentage of buffalo oocytes that reached full expansion was 67.4%, 57.4, 47.2 and 0.0% for excellent, good, fair and poor quality oocytes, respectively. Also, Barakat (2005) reported that oocyte quality affected cumulus expansion, where the excellent and good oocytes gave higher cumulus expansion than other grades. In this respect, a positive correlation between viability of hamster cumulus cells and ability to undergo expansion during maturation (Leibfried-Ruttledge et al., 1986) and between numbers of excellent and good quality oocytes and number of oocytes that showed full expansion (Abd El-Kader, 2005).

The recorded highest rate of cumulus cells expansion of the excellent oocytes was attributed to the number of layers of cumulus cells surrounding the oocytes. Good and fair quality oocytes had less number of cumulus cell layers and less percentage of cumulus cells expansion (Abdel-Razik, 2007). The existence of a healthy population of somatic cells surrounding the oocyte is mandatory to facilitate the transport of nutrient and signals into and out of the oocyte (Osborn and Moor, 1982). The role of cumulus cells in providing nutrients to the oocyte during its growth, to participate in the zona formation and following the LH surge to synthesize the matrix composed of proteins and hyaluronic acid was reported by Bedford and Kim (1993). The observed higher cumulus expansion may be due to the participation of oocytes for cumulus cells to synthesize hyaluronic acid and undergo cumulus expansion *in vitro* (Buccino *et al*., 1990).

Nuclear maturation with TCM-199 and DMEM:

Table (2) shows that the effect of medium type on nuclear maturation was significant only in fair and poor oocytes, being higher with DMEM than TCM-199 (51.5 *vs.* 26.8% and 32.8 *vs.* 16.1%). The differences in nuclear maturation of excellent and good oocytes between TCM and DMEM were not significant.

In comparable with the present results, maturation rate of bovine oocytes with different qualities was 45.3% (Smetanina *et al*., 2000), 62.7% (Oyamada *et al*., 2003), 78.2% (Luo *et al*., 2002) for DMEM versus 29.4 (Smetanina *et al*., 2000) and 76.8% (Abdel-Razik, 2007) for buffalo oocytes matured by TCM-199. In comparison TCM-199 with other media, Ali (2004) reported that the maturation percentage of buffalo oocytes using mSOF was (87.6%) and lower than that obtained by using TCM-199 medium with growth factors or hormones (93.9 - 96.1%). Similar results were reported by Barakat (2005) where TCM-199 medium exhibited higher maturation rate (77.6%) than mSOF medium (40.9%). Also, Raza *et al*. (2001) revealed that TCM-199 resulted in significant better maturation rate (73.3%) than Ham's 10

(61.6%). Similar results were obtained by Abdel-Razik (2007), who found that TCM-199 showed higher (P<0.01) maturation rate of buffalo oocytes than that of mSOF medium (76.8 *vs.*71.1%).

The difference in maturation percentage among different types of medium may be attributed to the composition of the media (Nandi *et al*., 2002). TCM-199 contains both glutamine and glucose (Michele *et al*. 2003). Presence of glucose is essential to generate ATP via glycolytic metabolism, while glutamine can feed into tricarboxylic acid cycle and serves as a potential energy source (Downs and Verhoeven, 2003). The absence of glucose or pyruvate fails to support the spontaneous meiotic maturation of mouse oocytes and does little more than help to maintain oocyte viability (Downs and Hudson, 2000). As affected by oocyte quality, excellent and good oocytes showed higher (P<0.05) maturation rate (76.7 and 70.9%) than fair and poor oocytes (40.2 and 24.6%), respectively. However, the differences between fair and poor oocytes were significant. In general, the highest maturation rate was obtained from excellent oocytes matured by DMEM (81.3%).

It is of interest to observe that poor oocytes did not reach full expansion showed low rate of neaclear maturation with both media, being higher for poor oocytes matured by DMED (32.8%) than fair oocytes matured by TCM (29.8%), reflecting the impact of DMEM on *in vitro* maturation of low quality oocytes (Table 2).

Resullts obtained in this study are similar to those reported by Leibfried-Ruttledge and First (1979) and Abdel-Razik (2007) for IVM of different oocyte qualities. Also, several authors found that there were marked differences in the rate of maturation of oocytes belonging to various morphological categories (Chain *et al*., 1995; Chauhan *et al*., 1998 and Abdoon *et al*., 2001). These differences indicate the substantial role of cumulus cells in oocyte maturity. In buffaloes, oocytes with homogenous cytoplasm and surrounded compact layers of cumulus cells showed significantly higher maturation rate than those with partial remnants or no cumulus cells (Warriach and Chohan, 2004).

Also, oocytes with expanded, clumbed cumulus cells complex and irregular cytoplasm showed lower rate of *in vitro* maturation (De Loose *et al*., 1989). On the other hand, Kim *et al*. (1997) reported no difference in developmental competence, even though the numbers of cumulus cell layers was low.

In general, cumulus cells are responsible for proper cytoplasmic maturation of the oocyte (Staigmiller and Moor, 1984 and Mori *et al*., 2000). Ravindranatha *et al*. (2002) revealed that oocyte maturation was mediated by the cumulus oophori and corona radiate surrounding the oocytes either cumulus expansion or nuclear maturation. Removal of cumulus mass adversely affected the developmental competence of oocytes (Nandi *et al*., 1998). Also, physical contact between oocyte and cumulus cells is necessary for the transfer of nutrients and other factors essential for oocyte development (Albertini *et al*., 2001). Cumulus cells keep the oocyte under meiotic arrest (Eppig, 1991) and participate in induction of meiosis (Mattioli and Barboni, 2000). Downs and Verhoeven (2003) concluded that cumulus cells enclosing the oocyte are responsible for the recognition and utilization of oocyte to the added compounds such as glucose and glutamine.

Effect of hormonal addition on *IVM*:

Cytoplasmic maturation:

Table (3) shows significant effect of hormonal addition to TCM-199 and DMEM on cumulus cells expansion of buffalo oocytes with different qualities. The highest percentage of oocytes showing full expansion was significantly (P<0.05) obtained when buffalo oocytes with different qualities (excellent, good and fair) were matured by DMEM supplemented with FSH. These findings may suggest the beneficial effects of hormonal addition on cumulus cells expansion for all oocyte qualities, except poor oocytes.

It is of interest to note that addition of FSH to TCM-199 yielded lower full expansion than that of FSH plus hCG addition, especially for excellent and good oocytes. Similarly, Abdel-Razik (2007) reported that the *in vitro* maturation rate of buffalo oocyte was lower with adding FSH than with FSH plus hCG to TCM-199 and mSOF media. Several authors reported that adding gonadotropins (LH, FSH or their analogues, singly or in combination) in maturation media usually had beneficial effects on oocyte maturation (Ravindranatha *et al.*, 2002, Chohan and Hunter, 2003; Ali, 2004 and Abd El- Kader, 2005).

The expansion of cumulus cells depends largely on the culture media used for maturation of the oocytes and supplementation of gonadotrophins in this media. In this respect, Nandi *et al*. (2002) found that the addition of gonadotrophins alone or with steroids caused significant increase in cumulus expansion.

In accordance with the obtained results for FSH addition to *in vitro* maturation medium on cumulus cells expansion, many authors suggested that FSH has beneficial effect on expansion of the cumulus cells surrounding the oocyte, which in terms enhance sperm capacitation and the fertilization process

(Eyestone and Boer, 1993 and Ravindranatha *et al*., 2002).

Expansion of cumulus cells surrounding bovine oocytes was altered in response to FSH and/or LH in semi-defined medium (Choi *et al*., 2001). It was explained that both FSH and hCG (mainly LH contaminated with FSH) increased the use of cAMP system as an intracellular second messenger (Bevers *et al*., 1997) and thus increases the level of activity of hyaluronic acid synthesis enzyme system and induced cumulus expansion in intact complexes (Buccino *et al*., 1990).

It was observed a direct role of FSH in bovine cumulus expansion which has been supported by the demonstration of receptor for FSH (Bao *et al*., 1997 and Kito and Bavister 1997). RT-PCR and *in situ* hyperidization experiments revealed that mRNA for FSH receptor was present in bovine cumulus cells but only few or no LH receptors. Thus, FSH is more effective in cumulus expansion at low concentration than LH (Elvin *et al*., 1999 and Choi *et al*., 2001).

On the other hand, Nandi *et al*. (2002) observed that cumulus cells were stimulated by gonadotropins (FSH and LH) to produce and secrete hyaluronic acid (Chen *et al*., 1990), causing dispersion of cells in a process called expansion and mucification. The presence of gonadotropin hormones in IVM media acts cooperatively to stimulate hyaluronic acid synthesis and maximal expansion of cumulus matrix (Chen *et al*., 1994).

Nuclear maturation:

Results presented in Table (4) show that hormonal addition to TCM-199 or DMEM also significantly affected IVM of buffalo oocytes. Maturation rate was significantly ($P<0.05$) the highest for excellent, good and fair oocytes matured by DMEM supplemented with FSH plus hCG. However, the poor oocytes showed the highest maturation rate ($P<0.05$) with DMEM supplemented with FSH alone.

Such trend may suggest that maturation rate as affected by hormonal addition was also in relation to oocyte quality, being the highest for the excellent oocytes matured by DMEM supplemented with FSH plus hCG and poor oocytes matured by DMEM supplemented with FSH alone. These results indicated that hormonal addition of FSH or FSH plus hCG to TCM-199 had no pronounced effect on in vitro maturation of buffalo oocytes with different qualities as compared to TCM-199. In accordance with the present results, Lawrence *et al*. (1980) reported that gonadotrophins act on cumulus cells by improving oocyte maturation *in vitro*. The prominent role of gonadotropins is the stimulation of nuclear maturation of oocytes (Totey *et al*., 1993). In this respect, Eppig (1991) reported that gonadotrophins, added *in vitro* to maturation media, enhanced bovine oocyte quality as shown by improved completion of nuclear maturation (95-100% matured oocytes), fertilization, and developmental ability. The role of gonadotropins in the maturation of oocyte has been widely acknowledged, and the experimental evidence of the resumption of meiosis has been reported when gonadotropins were added to the medium (Sanbuissho and Threfall, 1990; Olson *et al*., 1991 and Choi *et al*., 2001).

Plancha and Albertini (1994) suggested that contractile events associated with cytokinesis and polar body extrusion might be under hormonal influence. In this way, Bevers *et al*. (1997) revealed that both FSH and LH use cAMP system as an intracellular second messenger. The improving IVM of buffalo oocytes by FSH plus hCG may be attributed to that gonadotropins may stimulate cumulus cells of oocyte-cumulus complexes (COCs) to secrete a positive factor that could override arrest due to hypoxanthine and could trigger meiotic resumption (Choi *et al*., 2001). They also added that LH increased glycolysis and glucose oxidation in oocyte-cumulus complexes and glutamine oxidation in mature oocytes denuded after LH exposure.

Moreover, Zuelke and Brackett (1992 and 1993) demonstrated that one possible mechanism of gonadotropins enhanced oocyte maturation *in vitro* was to initiate changes in metabolism of COCs. Downs (1993) reported that gonadotrophins stimulate meiotic induction and led to generation of positive factor that acts on oocytes to override the inhibitory influence and to induce germinal vesicle breakdown (GVBD). The observed trend of differences in hormonal addition for TCM-199 or DMEM suggested that hormonal addition to *in vitro* maturation medium had different effect on both cytoplasmic and nuclear maturation. However, the slight differences in nuclear maturation may reflect the effect of FSH on nuclear maturation of oocyte and that was dependent on substrates present in IVM medium (Ali and Sirard, 2002).

CONCLUSION

Success *in vitro* maturation of buffalo oocytes is related to its qualities. Moreover, adding gonadotropins (FSH or FSH plus hCG) to maturation media improved cytoplasm and nuclear maturation. Further studies are needed for evaluation the effect of hormonal addition with FSH and/or hCG to DMEM medium on *in vitro* fertilization and embryo development of buffalo oocytes.

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Table 1. Effect of type of maturation medium on cumulus cells expansion of buffalo oocytes with different qualities

Oocyte Quality	Medium	N	Grade of cumulus expansion							
			G0		G1		G2		G3	
			n	%	N	%	n	%	n	%
Excellent	TCM	92	-	-	6	6.5	34	37.0	52	56.5
	DMEM	86	-	-	15	17.4	25	29.1	46	53.5
	Mean	178	-	-	21	11.8	59	33.2	98	55.1[a]
Good	TCM	62	2	3.2	8	12.9	26	41.9	26	41.9
	DMEM	107	-	-	23	21.5	36	33.6	48	44.9
	Mean	169	2	1.2	31	18.3	62	36.7	74	43.8[b]
Fair	TCM	72	12	16.7	14	19.4	36	50.0	10	13.9[b]
	DMEM	93	28	30.1	18	19.4	23	24.7	24	25.8[a]
	Mean	165	40	24.2	32	19.4	59	35.8	34	20.6[C]
Poor	TCM	84	56	66.7	14	16.7	14	16.7	-	-
	DMEM	86	66	76.7	20	23.3	-	-	-	-
	Mean	170	122	71.8	34	20.0	14	8.2	-	-

Means denoted within the same column with different superscripts are significantly different at P<0.05. N: Total number of oocytes. Grade 0 (Go): no expansion, Grade 1 (G1): few expansions of cumulus layers, Grade 2 (G2): moderate expansions of cumulus layers and Grade 3 (G3): full expansion of cumulus layers.

Table 2. Effect of type of maturation medium (TCM-199 vs. DMEM) on *in vitro* maturation rate of buffalo oocytes with different qualities

Medium	N	Immature oocytes				Mature oocytes						Degen. oocytes		MR (%)
		GV		GVBD		Anaphase		Telophase		M II				
		n	%	n	%	n	%	N	%	n	%	n	%	
Excellent oocytes:														
TCM	65	-	-	16	24.6	12	18.5	8	12.3	27	41.5	2	3.1	72.3
DMEM	64	-	-	12	18.8	12	18.8	9	14.1	31	48.4	-	-	81.3
Mean	129	-	-	28	21.7	24	18.6	17	13.2	58	45.0	2	1.6	76.7[a]
Good oocytes:														
TCM	45	-	-	7	15.6	9	20.0	6	13.3	14	31.1	9	20.0	64.4
DMEM	82	-	-	12	14.6	21	25.6	9	11.0	31	37.8	9	11.0	74.4
Mean	127	-	-	19	15.0	30	23.6	15	11.8	45	35.4	18	14.2	70.9[a]
Fair oocytes:														
TCM	56	10	17.86	23	41.1	1	1.8	1	1.8	13	23.2	8	14.3	26.8[b]
DMEM	66	5	7.58	18	27.3	10	15.2	8	12.1	16	24.2	9	13.6	51.5[a]
Mean	122	15	12.30	41	33.6	11	9.0	9	7.4	29	23.8	17	13.9	40.2[b]
Poor oocytes:														
TCM	56	14	25.00	18	32.1	-	-	1	1.8	8	14.3	15	26.8	16.1[b]
DMEM	58	7	12.07	13	22.4	13	22.4	2	3.5	4	6.9	19	32.8	32.8[a]
Mean	114	21	18.42	31	27.2	13	11.4	3	2.6	12	10.5	34	29.8	24.6[c]

Means denoted within the same column with different superscripts are significantly different at P<0.05. N: total number of oocytes, GV: germinal vesicles, GVBD: germinal vesicles breakdown, M II: metaphase II and Degen.: degenerated MR: Maturation rate

Table 3. Effect of hormonal addition to maturation medium (TCM-199 or DMEM) on cumulus cells expansion of buffalo oocytes with different qualities

Hormone	Medium	N	Grade of cumulus expansion							
			G0		G1		G2		G3	
			n	%	n	%	n	%	n	%
Excellent oocytes										
FSH	TCM	27	-	-	3	11.1	11	40.7	13	48.2[c]
	DMEM	29	-	-	-	-	6	20.7	23	79.3[a]
FSH+hCG	TCM	65	-	-	3	4.6	23	35.4	39	60.0[b]
	DMEM	57	-	-	15	26.3	19	33.3	23	40.4[c]
Good oocytes										
FSH	TCM	20	-	-	4	20.0	8	40.0	8	40.0[b]
	DMEM	37	-	-	4	10.8	8	21.6	25	67.6[a]
FSH+hCG	TCM	42	2	4.8	4	9.5	18	42.9	18	42.9[b]
	DMEM	70	-	-	19	27.1	28	40.0	23	32.9[bc]
Fair oocytes										
FSH	TCM	25	2	8.0	8	32.0	11	44.0	4	16.0[b]
	DMEM	36	-	-	2	5.6	16	44.4	18	50.0[a]
FSH+hCG	TCM	47	10	21.3	6	12.8	25	53.2	6	12.8[b]
	DMEM	57	28	49.1	16	28.1	7	12.3	6	10.5[b]
Poor oocytes										
FSH	TCM	21	15	71.4	2	9.5	4	19.1	-	-
	DMEM	40	20	50.0	20	50.0	-	-	-	-
FSH+hCG	TCM	63	41	65.1	12	19.1	10	15.9	-	-
	DMEM	46	100	-	-	-	-	-	-	-

Means denoted within the same column for each oocyte quality with different superscripts are significantly different at P<0.05. N: total number of oocytes, Grade 0 (Go): no expansion, Grade 1 (G1): few expansions of cumulus layers, Grade 2 (G2): moderate expansions of cumulus layers and Grade 3 (G3): full expansion of cumulus layers.

Table 4. Effect of hormonal addition to maturation medium (TCM-199 or DMEM) on maturation rate of buffalo oocytes with different qualities

Hormone	Med.	N	Immature oocytes				Mature oocytes						Degen. Oocyte		MR (%)
			GV		GVBD		Anaph.		Teloph.		M II				
			N	%	n	%	n	%	n	%	n	%	n	%	
Excellent oocytes:															
FSH	TCM	24	-	-	6	25.0	5	20.8	3	12.5	9	37.5	1	4.17	70.8^{b}
	DMEM	24	-	-	5	20.8	4	16.7	4	16.7	11	45.8	-	-	79.2^{ab}
FSH +	TCM	41	-	-	10	24.4	7	17.1	5	12.2	18	43.9	1	2.44	73.2^{b}
hCG	DMEM	40	-	-	7	17.5	8	20.0	5	12.5	20	50.0	-	-	82.5^{a}
Good oocytes:															
FSH	TCM	18	-	-	4	22.2	4	22.2	2	11.1	5	27.8	3	16.67	61.1^{b}
	DMEM	31	-	-	5	16.1	8	25.8	3	9.7	11	35.5	4	12.90	71.0^{ab}
FSH+hCG	TCM	27	-	-	3	11.1	5	18.5	4	14.8	9	33.3	6	22.22	66.7^{b}
	DMEM	51	-	-	7	13.7	13	25.5	6	11.8	20	39.2	5	9.80	76.5^{a}
Fair oocytes:															
FSH	TCM	24	4	16.7	11	45.8	1	4.2	-	-	5	20.8	3	12.50	25.0^{b}
	DMEM	28	3	10.7	6	21.4	4	14.3	3	10.7	7	25.0	5	17.86	50.0^{a}
FSH+	TCM	32	6	18.8	12	37.5	-	-	1	3.1	8	25.0	5	15.63	28.1^{b}
hCG	DMEM	38	2	5.3	12	31.6	6	15.8	5	13.2	9	23.7	4	10.53	52.6^{a}
Poor oocytes:															
FSH	TCM	20	5	25.00	6	30.0	-	-	1	5.00	2	10.00	6	30.00	15.0^{c}
	DMEM	29	3	10.34	7	24.1	7	24.14	-	-	4	13.79	8	27.59	37.9^{a}
FSH+	TCM	36	9	25.00	12	33.3	-	-	-	-	6	16.67	9	25.00	16.7^{c}
hCG	DMEM	29	4	13.79	6	20.7	6	20.69	2	6.90	-	-	11	37.93	27.6^{b}

Means denoted within the same column for each oocyte quality with different superscripts are significantly different at P<0.05. N: total number of oocytes, GV: germinal vesicles, GVBD: germinal vesicles breakdown, M II: metaphase and MR: maturation rate.

تاثير بيئات الإنضاج مع الإضافة الهرمونية على إنضاج بويضات الجاموس مختلفة الجودة معملياً

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تهدف هذه الدراسة تقييم تأثير أنواع مختلفة من بيئات إنضاج البويضات مع إضافة كلٌ من هرمون HCG, FSH على الإنضاج المعملى لبويضات الجاموس مختلفة الجودة. تم تجميع مبايض الجاموس وجمع البويضات بطريقة الشفط تم تصنيف البويضات على حسب عدد طبقات الخلايا الركامية المحيطة بالبويضة إلى ممتاز – جيد – مقبول – فقير. تم تحضين البويضات معمليا فى بيئات DMEM , TCM.199 لدراسة تأثير نوع بيئة الإنضاج وكذلك لدراسة تأثير الإضافة الهرمونية مثل إضافة هرمون FSHمع HCG إلى بيئات الإنضاج. تم تقييم البويضات على حسب نضج السيتوبلازم ونضج النواة. أظهرت النتائج تأثيرا معنويا لنوع بيئة الانضاج على نضج السيتوبلازم فقط بالنسبة للبويضات مقبولة الجودة وعلى نضج الانوية فى حالة البويضات المقبولة والفقيرة الجودة فقط وكان هذا التأثير أعلى مع بيئة DMEM عن TCM199 كما لوحظ ان هناك اختلاف غير معنوى فى درجة نضج الانوية بين بيئة DMEM وبيئة TCM199 كما ان البويضات الممتازة والجيدة كانت اعلى معنويا من البويضات المقبولة والفقيرة فى درجة نضج الانوية . بصفة عامة اظهرت البويضات ممتازة الجودة أعلى مستوى للنضج التى تم تحضينها فى بيئة DMEM كذلك كان اعلى نسبة للبويضات التى اظهرت تمدد كامل للخلايا الركامية ظهر فى البويضات الممتازة ،الجيدة ، المقبولة المحضنة فى بيئة DMEM والمضاف اليها هرمون FSH على التوالى. من هذه النتائج المتحصل عليها ممكن ان نتوقع تاثيرات مفيدة للمعاملة الهرمونية على تمدد الخلايا الركامية فى البويضات ذات الجودة المختلفة ماعدا البويضات الفقيرة كذلك يرتبط معدل نضج البويضات بمدى جودة البويضات حيث زاد هذا المعدل فى البويضات الممتازة يليها الجيدة ثم الفقيرة المحضنة فى بيئة DMEM مع اضافة هرمون FSH+HCG بينما اظهرت البويضات الفقيرة اعلى معدل نضج مع بيئة DMEM المضاف اليها هرمون FSH فقط . كما ان اعلى معدل لنضج الانوية كان ايضا فى البويضات ذات الجودة المختلفة المحضنة فى بيئة DMEM المضاف اليها هرمون FSH+HCG . وتوصى الدراسة بانة هناك حاجة لمزيد من الدراسة لدراسة تاثير الإضافات الهرمونية لبيئة الإنضاج على الإنضاج المعملى والتطور الجنينى لبويضات الجاموس.