

Outcome of Baghdad culture medium and in vitro fertilization of human eggs and embryo transfer outcome.

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Summary:

Background: *In vitro* fertilization and early embryo development require special medium which meet the minimal nutritional requirements.

Objective: To formulate a new culture medium (Baghdad Culture Medium BCM) to be used in *in vitro* fertilization and embryo transfer and to compare its efficiency with imported (IVFM).

Materials & Methods: In the present study, BCM was used in 52 couples and IVFM in 49 couples admitted in Baghdad culture in IVFM group and 49 couples admitted in IVFM group. Females had blocked tubes, ovulatory disturbances, or luteal phase defect. The males had normal seminal fluid parameters.

Result: The number of fertilized oocytes was significantly higher in BCM group compared to IVFM group. The rate of embryo development *in vitro* and the number of pregnancy rate were significantly higher in BCM group compared to IVFM group. No significant differences between both groups regarding the number of mature oocytes.

Conclusion: We conclude from the study that Baghdad culture medium gave better result than IVFM regarding fertilization rate, embryo development and pregnancy rate. This may be because BCM contains efficient and sufficient ingredients, and freshly prepared, which are important in human fertilization *in vitro* and embryo implantation.

Keywords: Baghdad culture medium (BCM), Human IVF-ET.

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Introduction

The media used for the transitional activities of follicular aspiration, and ovum maturation and fertilization must meet the minimal nutritional requirements of the oocytes(1). The media should provide appropriate pH stability through buffer actions that meet the osmotic needs of the oocytes and be compatible with culture system selected for insemination and embryo growth (2).

It has been known in experiment on mice that only pyruvate and oxaloacetate can support oocytes development in the absence of cumulus cells, but in their presence, lactate, phosphoenolpyruvate and glucose are able to support maturation and the first cleavage division (3). This finding was followed by direct demonstrations of the ability of cumulus cells to produce pyruvate by using glucose as a substrate (4). Most recent work has shown that fertilization and early development of the human embryo require little glucose(5). There seem to be no special ionic or amino acid requirement for oocytes in the transitional state, (the transfer of oocytes

from follicles to CO₂ incubator) and the electrolytic as well as the osmotic needs are met by most balanced salt solution.

The most important part played by transitional media is the prevention of pH shift. The selection of an appropriate natural buffer is, therefore the most critical decision in selecting culture media (3). The most popular buffer used in culture medium for assisted reproductive technique (ART) are ethanesulfonic acid (HEPES). This buffer system has the advantages of low toxicity to human oocytes and embryos (7). An equilibrium of carbonic acid (H₂CO₃) and bicarbonate ion (HCO₃) is reached at pH 7.4 when bicarbonate is included in medium in the presence of elevated atmosphere carbon dioxide (CO₂) (6).

The addition of human serum albumin (HAS) at concentration of 2 to 10 mg/ml in the medium suppresses stickiness and provide osmotic stability. The human culture medium which is used for follicular Hushing should be devoid from all protein as rare instances of anaphylaxis relating to HAS have been reported. Heparin is added to Hushing medium to flush the aspiration needle but is not included in the follicular flushing medium since it could impair clotting at the site of entry in to the ovary made by the aspiration needle. Flushing and holding culture media are incubated at

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37°C in 5%CO₂ incubator (7).

The objectives of the present study were to formulate a new culture medium (Baghdad culture medium : BCM) to be used in IVF work and to compare its efficiency in supporting human IVF rates and embryo development with another culture medium (IVFM) imported from Medicult Company from Denmark. The BCM was prepared in Saddam's IVF Institute.

Materials and Methods:

1. Patients:

The number of the patients was 52 in BCM group and 49 in IVFM. The age of the patients was 32.57 ± 1.30 years in BCM and 33.53 ± 1.44 in IVFM. The female partner had either blocked tubes, ovulatory disturbances, or luteal phase defects. The males had normal seminal fluid parameters and less than 10% of them had border line asthenosperma.

2 . IVF medium:

The IVF medium (Medicult Company) contains: 1) water, 2} balanced salt. 3) energy substrate (glucose, pyruvate and lactate). 4) proteins such as human serum albumin and synthetic serum replacement. 5) antibiotics such as penicillin and streptomycin. 6) heparin IOIU/ml, 7) aminoacids, 8) vitamins, 9) hormones and growth factors (insulin 0.5mg/L), 10) buffer system such as sodium bicarbonate/CC>2 HEPLIS. The IVFM was ready for use and its osmolality ofwas 285 ± 8 mOsm/Kg and a pH of 7.44.

3 . Baghdad culture medium, (BCM):

The Baghdad Culture Medium (BCM) contains Varies balanced salt (HBS) 884 mg per 100ml o' distill water, sodium pyruvate 8mg/100ml. sodium bicarbonate 210 mg/100ml. ampicillin 8mg/100ml 20% preovulator>' inactive maternal serum and 10% human follicular fluid (HFF). The water used for BCM preparation was distilled and filtered with 0.22 M|a filler. The BCM had a pH of 7.2-7.40 and osmolality of 285 ± 5 mOsm/Kg Ovulation induction and IVF were described else where (1). The morphological assessment of oocyte maturity is shown in table 1.

Results:

The clinical data of invitro fertilization (IVF) program using Baghdad culture medium (BCM) and IVF culture medium (IVFM) is shown in table number 2. The number of the patients in BCM group was 52 while the number of the patients in the IVFM group was 49. The number of ampoules used by each patient was higher in IVFM group compared to BCM group (22.90 ± 1.66 versus 21.26 ± 1.05 ampoules respectively). The number of stimulation days in the IVFM group was also longer than that in the BCM group (11.45 ± 0.35 days and 10.63 ± 0.32 days). The number of the oocytes that were recovered from the patients was 233 in BCM group and 237 in IVFM group. Table number 3 shows the number of oocytes retrieved per patient, which was similar in both groups.

The number of mature oocytes was 197 in BCM group and 194 in IVFM group. The number of fertilized oocytes was higher in BCM group compared to IVFM group (187 versus 180 respectively). The number of transferred embryos per patient was similar in both group (2.59 ± 0.11 in BCM group and 2.67 ± 0.23 in IVFM group). The number of pregnant patients was higher in BCM group compared to IVFM group (table 3).

The percentage of mature oocytes was similar in BCM and IVFM groups (84.54% in BCM and 81.85% in IVFM). Figure 1 shows the percentage of "mature oocytes 'following follicular aspiration and culturing of the human oocytes in Baghdad culture medium and IVF medium. Figure 2 shows the in vitro fertilization of mature human oocytes using BCM and IVFM as culture media. The percentage of the fertilized oocytes by conventional IVF (cIVF) was higher in BCM compared to IVF medium ($P < 0.05$. 80.25% versus 75.94%). Embryo developmental rate after in vitro fertilization of human oocytes was significantly higher in BCM ($P < 0.01$) compared to IVF medium (77.74% versus 65.00% respectively. Fig. 3). The pregnancy rate in BCM was also significantly higher when compared to IVF medium ($P < 0.05$. Fig. 4). The pregnancy rate after cIVF was 34.61% in BCM group while in IVFM group was 28.75%.

Table (1) Morphologic parameters used for assessment of oocyte maturity at Saddam IVF Institute for Embry Research and Infertility Treatment at University of Baghdad

Grade of oocyte maturity	Oocyte	Associated granulose	Cumulus oophorus	Corona radiate
Immature oocytes (Prophase I, unripened oocyte)	Germinal vesicle present	Aggregated; compact cells	Dense	Compact
Intermediate oocyte (metaphase I)	No germinal vesicle; no first polar body	Loosely aggregated	Slightly expanded	Expanded corona
Mature oocytes (metaphase II, preovulatory)	First polar body present	Sparse	Expanded; dispersed cells	Expanded; radiant (sunburst)
Atretic oocytes	Very dark; difficult to identify	Fragmented; lace-like identify	Absent	Absent

Table (2) the clinical data of the in vitro fertilization (IVF) program using Baghdad culture medium (BCM) and universal IVF culture medium (IVFM)*

Parameters *	BCM	IVFM
Number of patients	52	49
Age of patients	32.57±1.30	35.53±1.44**
Number of ampoules used by patient	21.26±1.05	22.90±1.66**
Stimulation days	10.63±0.32	11.45±0.35**

* Data are Mean ± standard error of mean (SEM)
 ** P> 0.05 non significant

Table (3) The clinical outcome of in vitro fertilization (cIVF) program for treatment of infertile patients using Baghdad culture medium (BCM) and universal IVF medium (IVFM)*

Parameters	BCM	IVFM
Number of oocytes recovered	233	237
Number of oocytes retrieved per patient	4.48±0.31	4.83±0.67
Number of mature oocytes	197	194
Number of fertilized oocytes	187	180
Number of transferred embryos per patient	2.59±0.11	2.67±0.23
Number of pregnant patients	18/52	14/49

* Data are Mean ± standard error of mean (SEM)

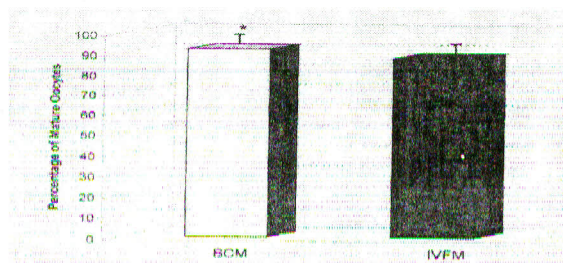


Figure (1). The percentage of human mature oocytes following follicular aspiration and culturing in Baghdad culture medium (BCM) and IVF medium (IVFM) in conventional IVF program.

*P<0.05: Not significant.

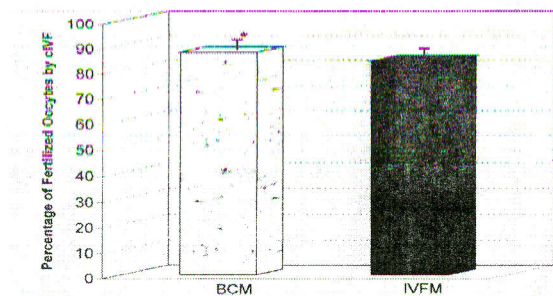


Figure (2). In vitro fertilization rate of human oocytes using Baghdad culture medium (BCM) and IVF medium (IVFM).

*P<0.05 Significantly different from other group.

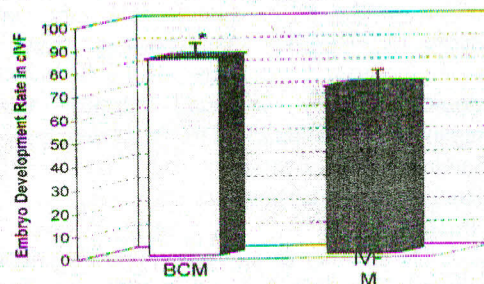


Figure (3). Embryo development rate after In Vitro Fertilization (IVF) of human oocytes using Baghdad culture medium (BCM) and IVF culture medium (IVFM).

*P< 0.01 Significantly different from other group.

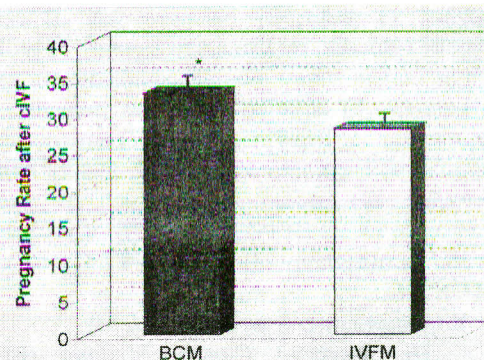


Figure 4. Pregnancy rate after In Vitro Fertilization (cIVF) of human eggs using Baghdad culture medium (BCM) an IVF culture medium (IVFM).

*P< 0.05 Significantly different from other group.

Discussion:

Oocytes retrieval rate were similar in both groups (BCM and IVFM). Numbers of mature oocyte were also similar in both groups. Harrison et al (8) and Strehler et al., (9) reported higher number of oocytes retrieved than that of BCM group, but all reported lower number of mature oocytes than BCM. This may be due to the use of different protocols for ovulation induction and high doses of gonadotropins, that lead to early growth of follicles and increased the total number of immature oocytes prior to follicles aspiration. Other researchers also (10) reported higher number of retrieved oocytes and mature oocytes than that of BCM. This may be because they used GnRIIa (Leuprolide acetate) at midluteal phase of previous cycle and induced ovarian stimulations by high dose of hMG from cycle day two. This leads to high estradiol concentration and higher number of

follicles on the day of hCG administration which leads to increase number of total oocytes retrieved as well as mature oocytes.

Fertilization rate in BCM was significantly higher than that of IVFM. Other workers reported similar results of fertilization rate but low oocyte retrieval (2.8 vs 4.4) than BCM (11). Perri, (12) reported similar in vitro fertilization rate to BCM by using different medium (PI medium supplemented with 10% serum substitute). It seems that the presence of human follicular fluid and serum in the BCM have stimulatory effect on activation of in vitro fertilization potential of human sperm (13). The human follicular fluid and mid-preovulatory serum are absent in IVFM and may be for this reason the IVF rate was low when compared to BCM in the present study.

The embryonic developmental rate in BCM was significantly higher ($P < 0.01$) than IVFM. Lower in vitro human embryonic development was reported when IVFM was used as a culture medium for the growth of human IVF embryos although different ovulation induction regimens were used (1). The superiority of BCM over IVFM in embryonic developmental rate may be related to the efficiency of the BCM to support embryonic development in vitro. The presence of gonadotropin and steroid hormones in the follicular fluid may play an important stimulatory effect on the cleavage of these embryos (4).

Conaghan, (15) reported high embryonic developmental rate in medium containing pyruvate compared to low developmental rate in medium containing glucose. Other workers (16) reported higher cleavage rate with medium containing human serum than that containing albumin. It has been reported that improvement in human embryo development was obtained by reducing glucose and phosphate levels from the medium and this corresponds with the result of this study in BCM, which was glucose free medium (17,18).

Chen et al., (2001) reported no significant difference in embryonic developmental rate between their medium and IVFM medium, but they reported higher pregnancy rate in IVFM than XI-HTF with 10% HAS (22.8% vs 14.2%). Early human embryo growth in vitro requires pyruvate for their normal metabolism. Improvement in embryo cleavages and increased in implantation rates were observed by using medium containing high concentration of human serum (9).

The number of transferred embryos was similar in BCM and IVFM, but pregnancy rate was significantly higher in BCM than IVFM. Sharma (20) reported similar

results of pregnancy rate to that of BCM, while Farm" (21) reported higher number of embryos than that of BCM but pregnancy rate was similar to that of BCM.

Strehler (9) reported similar results of embryo transfer, but with lower pregnancy rates than BCM. BCM contains human serum with high viscosity, which helps embryo implantation better than serum-free media. Other workers reported similar results to BCM in regard to the number of embryo transfer, but with lower pregnancy rate than BCM (22,23). These differences in the result may be due to the differences in age groups studied by these workers, which were older than that of BCM. Dicker (24) reported that age of patient affect pregnancy rate and increase in age leads to poor pregnancy rate. Picette (25) also found that increase maternal age leads to decrease in pregnancy rate.

Baghdad culture medium (BCM) gave better result than IVFM in regard to fertilization rate, embryonic development, and pregnancy rate. This may be because BCM contains sufficient and efficient nutritive ingredients and stimulatory factors such as energy substrate, preovulatory inactive serum factors in human IVF, embryo development and implantation (16,17,19,26-28).

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