THE EFFECT OF DIFFERENT CULTURE MEDIA ON *IN VITRO* FERTILIZATION OF MICE

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Abstract

The effect of different types of media on *in vitro* fertilization (IVF) of mice oocyte was investigated. IVF was carried out in RPMI-1640 medium at recommended temperature (37º C) and pH (7.4). The obtained results showed that 63.73% of used oocytes were fertilized, and the percentage of abnormal embryos was 27.58%. While, using other culture media at same temperature and pH, gave low percentage of fertilization and higher yield of abnormal embryos. Thus, using Medicult IVF, Earl's and TCM-199 were gave fertilization percentages equivalent to 38.75, 42.42 and 61.45% respectively; whereas, the percentages of abnormal embryos were 32, 35.7 and 33.89 % respectively.

The reported results indicate that composition of various types of culture media have significant effect on the rate of fertilization and abnormal embryos.

Keywords: culture medium, *in vitro* fertilization, IVF.

Introduction

There are several environmental factors which might influence IVF of mammalian oocytes, such as temperature, type of medium, pH, gas phase and other factors. Mammalian Cells can only exist outside their natural in vivo surroundings, only if the *in vitro* environment mimics that of living body, in this respect culture media are used to provide the chemical and physiological elements which cells need to survive *in vitro* [1]. Successful animal and human IVF is dependent on using of suitable cultural conditions for gametes and early embryo's development, thus, various investigators have tried a number of different media and cultural conditions. Media used for IVF have ranged from simple buffered balanced salt solutions [2] to more complex media supplemented with amino acids and various cofactors [3, 4]. Supplementation of media with different exogenous protein sources (e.g. serum albumin and fetal calf serum) yield variable effects for rate of IVF and embryo development [5, 6], indicating that optimal cultural conditions for supporting IVF and embryo development have not yet attained.

The purpose of this investigation was to investigate an *in vitro* fertilization rates and the yield of abnormal embryos in various types of media.

Materials and Methods

Culture Medium

RPMI-1640, Medicult IVF, Earl's and TCM-199 were used.

Animal and animal care:

Healthy adult Swiss albino mice were obtained from animal house of Al-Nahrain Research center for Biotechnology. 180 mice were used in this study (140 female and 40 male), the age of the mice were in the range of 3.5 to 4 months old, and the weight in the range 25-30 grams. The animals were housed in small plastic cages, which were cleaned weekly by washing with soap and tap water and sterilized with 70% ethyl alcohol through out the period of the study. The room temperature was maintained at 24º±2º C, and the animals were exposed to 14 hours light programme [7].

Oocytes collection and maturation

The female was superovulated by intraperitonial injection with 10 IU of pregnant mare serum gonadotrophin (Folligon), followed after 48 hours with 10 IU hCG [8]. After 16 hours of hCG injection, the females were sacrificed and their reproductive

organs were excised and washed with RPMI-1640 under sterile conditions. Then under the stereomicroscope the oocytes were collected from the oviducts by flushing with RPMI-1640. According to morphological features, oocytes were classified into immature, mature and atretic.

Oocytes maturation was carried out by placing the collected oocytes in sterile Petri dishes containing RPMI-1640 media and incubated in a CO2 incubator at 37 ºC with 5% CO2 in humidified air for at least four hours.

Sperm collection and in vitro fertilization

After sacrificed male mice and both vas deferens were excised and washed by culture medium, then sperm were collected by flushing of vas deference by sterile syringe G-28 containing 1 ml of RPMI-1640 from one side of vas deference and collect the sperm from the other side of vas deference [9]. The sperms were then added to oocytes and incubated at 37º C in humidified CO2 incubator. Twenty four hours after insemination, percentages of fertilization and embryonic development were assessed.

Effect of type of media on in vitro fertilization

Various types of media were used in this study, RPMI-1640, TCM-199, Medicult and Earl's-TCM199 to ascertain the effect of medium composition on *in vitro* fertilization and embryonic development.

Statistical analysis

The obtained data were statistically analyzed by using Chi square test to compare between different values to observe the level of significance (0.05) [10].

Results and Discussion

Different types of media were used for assessment of *in vitro* fertilization of mice oocytes including Medi-Cult IVF medium, Earl's-TCM-199, TCM-199 and RPMI-1640. The obtained results (Fig.(1)) of the IVF experiments which were carried out in RPMI-1640 medium at optimum temperature (37º C) and pH (7.4), showed that 63.73% of used oocytes were fertilized, and the percentage of abnormal embryos was 27.58%. On the other hand at the same temperature and pH, Medicult IVF, Earl's-TCM-199 and TCM-199 culture media gave fertilization percentages equivalent to 38.75%, 42.42 and 61.45 respectively; whereas, the percentages of abnormal embryos were 32, 35.7 and 33.89 % respectively Fig.(2). The reported results showed that the percentage of in vitro fertilization was significantly ($P<0.05$) increased when mice oocytes incubated in RPMI-1640 medium as compared with the other culture media used in present work. Moreover, the least percentage of embryonic abnormalities was observed within RPMI-1640. However, no significant differences ($P>0.05$) were noticed in the percentages of fertilization and embryonic abnormality for other culture media (Medi-Cult IVF, Earl's-TCM-199 and TCM-199). Although, better results were obtained in Medi-Cult IVF medium in comparison with Earl's-TCM-199 and TCM-199 media, this might be due to presence of complex protein source (human serum albumin and synthetic serum replacement) in the basic formula of Medi-Cult medium [11]. It was reported that the fertilization and growth of embryos within culture medium is dependent on the basic constituents of the medium as well as additives [12]. In the present study, the chemical composition of RPMI-1640 proved superior in comparison with other media. The components of this medium include amino acids, glucose, vitamins and different organic and inorganic compounds. Presence of glucose (4.5 g/L) in RPMI-1640 is considered an excellent factor because it has important role in sperm capacitation [13]. The available published papers have never mentioned the importance of this medium in *in vitro* fertilization.

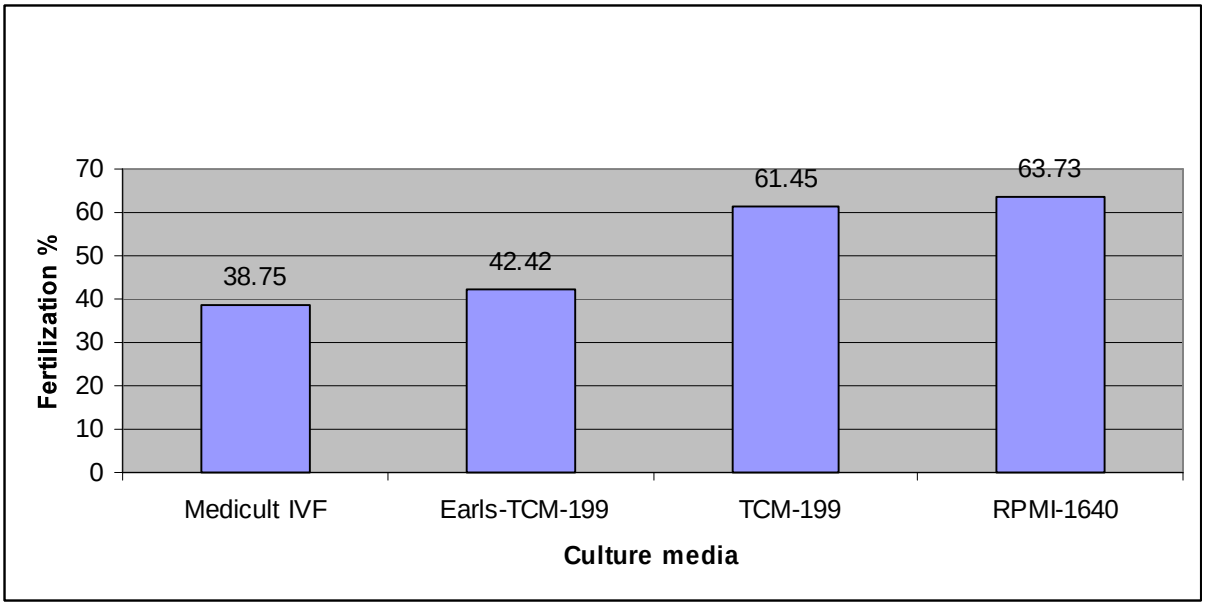

Fig. (1) : The effect of culure media on percentage of in vitro fertilization.

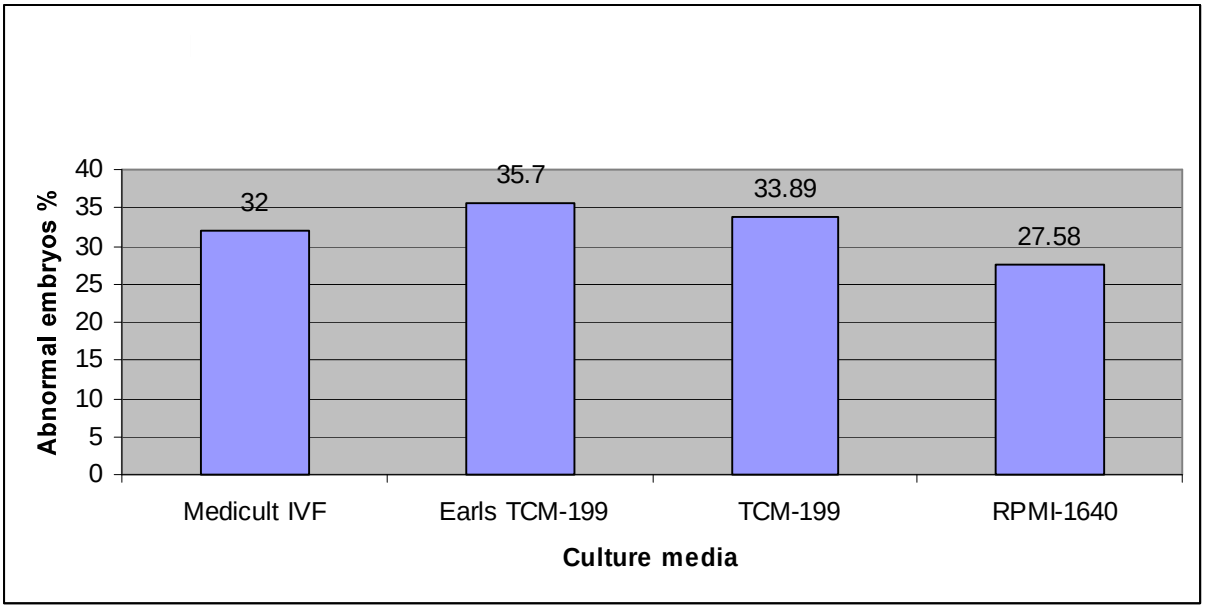

Fig. (2) : The effect of culure media on percentage of abnormal embryos development.

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الخلاصة

ان دراسة تاثير الاوساط الزرعية علـى الاخـصاب الخارجي في الفئران قـد اوضـحت سـابقا . الاخـصاب الخارجي قد تم باستخدام وسـط RPMI-1640 وبدرجـة الحرارة الموصى بها وهي 37° م والحامضية PH(7.4) . اظهرت النتائج ان 63.73% من البيوض المـستخدمة قـد خصبت وان نسبة الاجنة المشوهة كانـت 27.58% بينمـا استخدام اوساط زرعية اخـرى وبـنفس درجـة الحـرارة والحامضية اعطت اقل نسبة اخصاب وزيادة في تـشوهات الاجنة . لذلك ان استخدام وسط IVF Medicult وEarls و TCM_199 اعطـت نـسبة اخـصاب 38.75% ، 42.42% و 61.45% على التوالي بينما كانت نسبة الاجنة المشوهه 32% ، 35.7% و 33.89% على التوالي .

نتائج البحث اثبتت ان مكونـات الاوسـاط الزرعيـة المختلفة الانماط (الانواع) قد أثرت وبشكل معنـوي علـى معدلات الاخصاب وانتاج أجنة مشوهه.

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