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In vitro fertilization (IVF) and development of mouse embryos

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ABSTRACT F1 female mice of 3-4 weeks old derived from the CBA/ca x C57BL/6J strain were superovulated with intraperitoneal injections of 5 I.U. of PMSG and HCG 48 hours apart. At 15 hours after HCG, the oocytes were fertilized with caudal epididymal sperm obtained from males of 10-15 weeks old of CBA/ca x C57BL/6J strain. The insemination concentrations were between 1-2 million sperm cells per mL of medium. At five hours after insemination, the oocytes were washed through a few series of medium changes to remove excess sperm and cumulus cells. The fertilized oocytes were cultured in an incubator in humidified 5% CO_2 in air at 37°C. Embryonic development was recorded until the blastocyst stage. Based on 2-cell stage embryos, fertilization rate of 90.7% was obtained and of the proportion of 2-cell stage embryos, 97.6% developed to the blastocyst stage.

ABSTRAK Mencit betina F1 berusia 3-4 minggu diperolehi daripada strain CBA/ca x C57BL/6J disuperovulasi dengan suntikan intraperitoneum dengan 5 I.U. PMSG dan HCG dengan jarak 48 jam. 15 jam selepas HCG, oosit desenyawakan dengan sperma kauda epididimis daripada jantan yang berusia 10-15 minggu daripada strain CBA/ca x C57BL/6J. Kepekatan inseminasi ialah di antara 1-2 juta sel sperma per mL medium. Lima jam selepas inseminasi, oosit dibasuh melalui beberapa siri pertukaran medium untuk membuang sperma dan sel kumulus berlebihan. Oosit yang disenyawakan dikultur di dalam suatu inkubator diwapair 5% CO_2 dalam udara pada suhu 37°C. Perkembangan embrio direkod sehingga ke peringkat blastosis. Berasaskan embrio peringkat 2-sel, kadar persenyawaan 90.7% diperolehi; dan daripada embrio peringkat 2-sel, 97.6% berkembang ke peringkat blastosis.

(*in vitro* fertilization, *in vitro* culture, mouse embryo development)

INTRODUCTION

Mammalian *in vitro* fertilization (IVF) has been a relatively recent advance. The first successful IVF was in the rabbit [1]. Subsequently, Whittingham [2] reported the first successful IVF in mice. The results from these pioneer studies have led to further success of IVF procedures in livestock animals particularly in cattle [3]. IVF is today the recognized treatment for infertility in man [4]. In human IVF, the procedure has successfully circumvented the problems of oligospermia, asthenospermia, autoimmunity and hostile cervical mucus [5]. It also has the potential to allow techniques such as gene therapy to be applied to human [6,7,8].

The IVF technique in mice has several applications. It provides a convenient way of obtaining a large number of oocytes and embryos for experimental studies including embryo transfer, cryopreservation and genetic manipulation (transgenism by nuclear gene microinjection and use of embryonic stem cells and cloning) [9]. The application of these techniques other than the traditional approach of artificial insemination will further accelerate the rate of propagation of commercially desirable traits in domesticated animals. Another practical application of mouse IVF is that it is one of the methods for quality control for checking culture medium and culture conditions in a human IVF-ET programme [10].

The successful implementation of these applications is dependent on a sound IVF procedure. Hence, the importance of establishing a reliable and consistent IVF procedure is a must. The objective of this study was to describe a routine procedure of fertilizing mouse oocytes obtained from superovulated mice.

MATERIALS AND METHODS

Mouse strains The oocytes were obtained from 3-4 weeks old F1 females (CBA/ca x C57BL/6J). Embryos derived from hybrid females are known for its vigour which is reflected in the embryonic development [11]. A group of 10-15 weeks old F1 males from the same strain was used as the sperm donors.

Superovulation The female mice were superovulated with intraperitoneal injections of 5 I.U. PMSG (Folligon, Intervet). This was followed 48 hours later by 5 I.U. HCG (Chorulon, Intervet).

Preparation of culture and washing medium Oocytes were fertilized in modified TYH medium [12]. Fertilized oocytes were cultured in modified WM [13]. Modified hepes-buffered WM was used as a washing medium. BSA was added to the media 1 day before use. All media were filter-sterilized through a 0.22 µm syringe filter (Millipore). Osmolarity for media was chec-

ked and adjusted to 274-295 mOsmole.

Preparation of fertilization and culture dishes 0.5 mL modified TYH medium was pipetted into one of the wells of a 4-well multidish (Nunclon, Denmark) for the sperm suspension. Tissue culture dishes (60 x 15 mm, Falcon) were used to prepare the fertilization dishes. TYH medium was pipetted in 200 µL drops and overlaid with silicone oil. Oocytes were fertilized in these droplets of medium. The fertilization and culture dishes were equilibrated in a CO_2 incubator in an atmosphere of 5% CO_2 in air at 37°C a day before use.

Recovery of sperm A mature male of 10-15 weeks old was despatched by cervical dislocation. The caudae epididymi were slit with a pair of fine scissors and the sperm were collected with a fine probe into the sperm suspension medium (modified TYH). The sperm suspension was preincubated in the CO_2 incubator for 1 hour before insemination.

Recovery of oocytes The superovulated female mice were sacrificed at 15 hours after HCG. Both oviducts were excised and placed into the silicone oil overlay in the fertilization dishes. A sharp probe was used to puncture the swollen ampulla. The cumulus mass that was released was immediately drawn into the fertilization drop.

Insemination of oocytes The freshly collected oocytes were inseminated with preincubated sperm in concentrations of 1-2 million sperm cells/mL of medium. The oocytes-sperm mixture was incubated for 5 hours after which the oocytes were washed with modified hepes-buffered WM to remove excess sperm cells and cumulus cells.

Embryo culture The fertilized oocytes were cultured in modified WM and subsequent embryo development was observed at regular intervals under an inverted microscope. Embryo cultures were terminated at 120 hours of incubation.

RESULTS

The average yield of oocytes per female was 27.1 (Table 1). The cleavage rate of fertilized oocytes was 90.7% (based on 2-cell stage development, Table 2). The proportion of 2-cell embryos which developed to the 4-cell, morula and blastocyst stages was 99.6%, 98.2% and 97.6% respectively (Table 3). The results showed good viability of *in vitro* fertilized embryos to survive culture conditions. Figure 1 shows the whole sequence of embryonic development at 24 hours intervals.

Table 1. *In vitro* mouse embryonic development after IVF.

Replicate	No. of female	No. of ova	2-cell	4-cell	Morula	Blastocyst
1	4	73	57	56	50	49
2	8	176	163	162	160	158
3	9	321	276	275	274	272
4	10	271	267	267	266	266
Total	31	841	763	760	750	745
Average		27.1				

Table 2. Percentage development of embryos from IVF.

Stage of development	Total no. (ova/embryos)	% development
Oocytes	841	100
2-cell	763	90.7
4-cell	760	90.3
Morula	750	89.1
Blastocyst	745	88.5

Table 3. Percentage embryo survival *in vitro* from 2-cell to blastocyst stage.

Stage of development	Total no. of embryos	% development
2-cell	763	100
4-cell	760	99.6
Morula	750	98.2
Blastocyst	745	97.6

DISCUSSION

The efficacy of the protocol described in this study is obvious from the results which showed a high fertilization rate and excellent embryonic development to the blastocyst stage.

In order to obtain a consistently high rate of fertilization, some consideration has to be given to the age and the strain of mouse used. Mice of more than 10 weeks old when induced to superovulate will yield a higher number of abnormal oocytes thus rendering fewer number of fertilizable oocytes [R.B. Abdullah, unpublished data].

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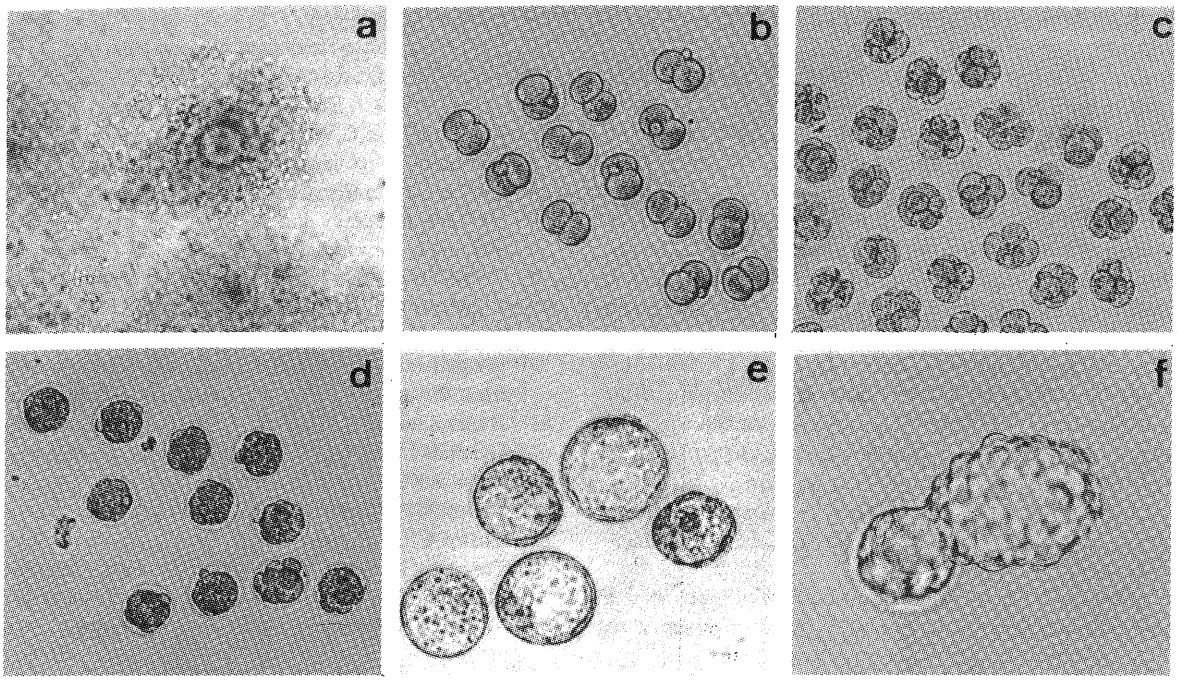

Figure 1. Sequence of mouse embryonic development.
a: Freshly ovulated oocytes; b: 2-cell embryos; c: 4- and 8-cell embryos; d: Compacted morulae; e: Expanded blastocysts; f: Hatching blastocyst. a-d, 100x magnification; e-f, 200x magnification.

Another factor that determines the fertilization rate and embryonic development is the strain effect. Successful embryonic development to the blastocyst stage is dependent on the culture medium requirements which is strain specific [14,15,16,17].

In our laboratory, the strain of choice is CBA/ca x C57BL/6J. We have found that modified TYH and modified WM are excellent fertilization and culture medium respectively. Both media were supplemented with 1 mM of L-glutamine and 1 mM taurine. Taurine is present at relatively high concentrations in the female reproductive tract fluids of several mammals [18,19,20]. The inclusion of taurine in the culture medium significantly promoted the development of 2-cell embryos to the blastocyst stage [21]. Supplementation with glutamine was beneficial in the first 48 hours of culture [22]. Without supplementation of glutamine and taurine, the fertilization and embryonic development was comparatively lower at 54.6% and 20% respectively in a previous study [R.B. Abdullah, unpublished data].

In conclusion, the IVF procedure developed in our laboratory is reliable and useful to other techniques. It also serves as the basic animal model for further studies in livestock animals and human reproduction.

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