DEVELOPMENT OF DIFFERENT STAGES OF ISOLATED BLASTOMERES AND ITS CORRESPONDING BIOPSIED EMBRYOS IN MICE

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Embryo splitting or blastomere separation has been successfully established since 1950s (1, 2). The blastomeres are developed to blastocysts where the ICM will form a pluripotent cells called embryonic stem cells (ESC) when cultured on mouse embryonic fibroblast (MEF) (3). The aim of this study was to produce blastocyst derived from isolating single blastomere at 2-, 4- and 8-cell stage in mice as model animal for later research in farm animals. A total of 40 ICR adult female mice were superovulated using 10 IU injection of pregnant mare serum gonadotrophin (PMSG, Folligon; Intervet) followed by another 10 IU injection of human chorionic gonadotrophin (hCG, Chorulon; Intervet) after 48 hrs and directly mated with ICR males (8-10 weeks old). The 2-cell embryos were collected from mated females by cervical dislocation at 48 hr post-hCG injection (4) and flushed in the hepes buffered mKSOM. The 2-cell flushed embryos were then cultured in mKSOM medium under silicon oil at 37°C in 5% CO_2 incubator until 4- or 8-cell stage before their blastomeres were separated. Microsurgical biopsy technique was used to isolate a single blastomere at 2-, 4- and 8-cell stages. The isolated single blastomeres and biopsied embryos were cultured in mKSOM under silicon oil at 37°C in 5% CO_2 incubator for further development until blastocyst stage.

Table 1 shows a higher successful rate of blastomere separation at 4-cell (75.9%) stage followed by 2-cell (72.3%) and 8-cell (53.7%). Table 2 shows the developmental rate of blastocyst formation in single blastomeres and biopsied embryos in which 4-cell stage gave the highest percentages for both single blastomeres and biopsied embryos groups. Figure 1 and Figure 2 show the development of single blastomeres and biopsied embryos, respectively.

In conclusion, single blastomere from early stage embryos can develop to blastosysts that can be used as a source of ICM for ESC line production although the percentage is low.

Table 1. Successful rate of blastomere separation by microsurgical biopsy.

Stage of embryos	No of embryo used for blastomere biopsy	Successfully separated (%)	Unsuccessfully separated (%)
2-cell	65	47 (72.3)	18 (27.7)
4-cell	58	44 (75.9)	14 (24.1)
8-cell	41	22 (53.7)	10 (46.3)

Table 2. The developmental rate of blastocyst formation in biopsied embryo and single blastomere

Stage of embryo	Single blastomere/ biopsied embryo	No. of single blastomere and biopsied embryo cultured	No. of blastosyst formed (%)
2-cell	Single blastomere	45	16 (35.6)
	Biopsied embryo	45	43 (95.6)
4-cell	Single blastomere	41	16 (39.0)
	Biopsied embryo	41	41 (100.0)
8-cell	Single blastomere	22	7 (31.8)
	Biopsied embryo	22	20 (90.9)

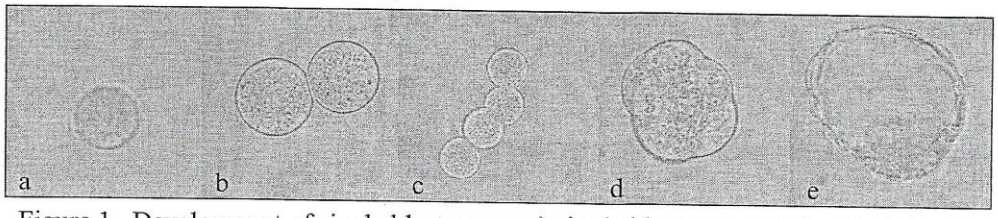

Figure 1. Development of single blastomere: a) single blastomere; b) 2-cell; c) 4-cell; d) morula; e) blastocyst

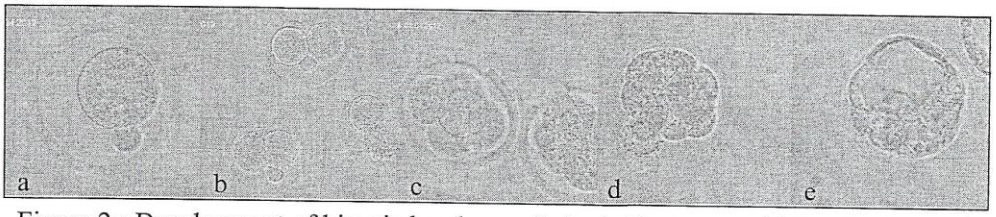

Figure 2 : Development of biopsied embryo: a) single blastomere; b) 2-cell; c) 4-cell; d) morula; e) blastocyst.

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