

The development of one- and two-cell mouse embryos in the absence of human serum

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Summary

One- and two-cell embryos were obtained from F1 hybrid female mice stimulated with human menopausal gonadotrophin and randomly distributed into two groups — group 1 (no serum) and group 2 (10% patient's serum). Fifty of 53 (94,3%) one-cell embryos in group 1 had cleaved to the blastocyst stage and 44 of 49 (89,79%) in group 2 after 96 hours (no significant difference — chi-square test). In the two-cell embryos in group 1, 78 of 89 (87,6%) reached the blastocyst stage after 72 hours and 80 of 86 (93,02%) in group 2 (no significant difference — chi-square test). According to microscopic evaluation cleavage to the blastocyst stage without serum supplementation is possible. The value of serum is discussed.

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The necessity of serum in culture media for culturing one-cell mouse embryos is debatable.¹ Cholewa and Whitten² have shown that the two-cell embryo will develop to the blastocyst stage in the absence of a fixed exogenous nitrogen source. An experiment was designed to compare the cleavage of one- and two-cell embryos simultaneously in Ham's F10 medium containing human serum and in medium containing no serum at all.

Materials and methods

F1 hybrid female mice (C57 B1/6 x CBA) were treated with 10 IU human menopausal gonadotrophin (HMG) followed by human chorionic gonadotrophin (HCG) 45 hours later to achieve super-ovulation. At the time of injection of HCG the females were mated with singly housed F1 hybrid studs. The females were sacrificed 45 hours later to obtain two-cell embryos.

To synchronize the time of obtaining one-cell embryos with the two-cell embryos another batch of F1 hybrid female mice were injected one day later with HMG; 45 hours later the HCG injection was given and the females were mated with the studs. Eighteen hours after the HCG injection the females were sacrificed and one-cell fertilized embryos were obtained at the pronuclear

stage by a standard technique.³ The one-cell embryos were pooled in a Petri dish (Falcon 3037), rinsed with 0,1% hyaluronidase to remove the cumulus oophorous cells and again rinsed twice in Ham's F10 medium (pH 7,4). Thereafter the fertilized embryos were distributed into 100% Ham's F10 medium (group 1) and the Ham's F10 supplemented with 10% human serum (group 2).

The two-cell embryos were also distributed into two groups in separate Petri dishes containing no serum (group 1) and 10% serum (group 2). The one-cell embryos were incubated in a 5% CO₂-in-air incubator for 96 hours and the two-cell embryos for 72 hours. The osmolarity of the medium was 280 mOsm/kg (Wescor Inc. 5100C). The medium used in the experiment had a pH of 7,4 (pH meter 83 Autocal).

The experiment was repeated twice. The percentage of embryos which cleaved to the blastocyst stage was evaluated by one of the authors (T.F.K.) without knowing to which group each embryo belonged.

Results

Of the one-cell embryos 94,33% cleaved to the blastocyst stage in group 1 and 89,79% in group 2 (Table I). The percentage cleavage among the two-cell embryos was 87,64% in group 1 and 93,02% in group 2 (Table II). Using the chi-square test for homogeneity, no statistically significant difference was found between the percentage cleavage in the one-cell embryos ($P = 0,39$) and in the two-cell embryos ($P = 0,23$).

TABLE I. CLEAVAGE OF ONE-CELL MOUSE EMBRYOS TO BLASTOCYST STAGE

Experiment	Group 1		Group 2	
	No.	%	No.	%
1	32/33	96,97	27/30	90,00
2	18/20	90,00	17/19	89,47
Total	50/53	94,34	44/49	89,79

TABLE II. CLEAVAGE OF TWO-CELL MOUSE EMBRYOS TO BLASTOCYST STAGE

Experiment	Group 1		Group 2	
	No.	%	No.	%
1	54/61	88,52	51/56	91,07
2	24/28	85,71	29/30	96,67
Total	78/89	87,64	80/86	93,02

Discussion

According to these results cleavage of one- and two-cell embryos is possible without serum supplementation. Cholewa and Whitten² have shown quite conclusively that two-cell

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embryos will develop to the blastocyst stage in the absence of a fixed nitrogen source. Furthermore they obtained viable young after transferring blastocysts grown in this medium.²

On the other hand, Fishel and Surani⁴ reported that the metabolism of the early stage embryo is unaffected by the absence of serum macromolecules, but at later stages of development the omission of such substances is apparent. The embryo will cleave and appear morphologically normal with or without the addition of serum, but the metabolism of such embryos may be affected.⁴ Saito *et al.*⁵ also stated that serum supplementation is required for embryo growth. The omission of serum can lead to chromosomal aberrations.

Other beneficial effects of the serum or albumin are the chelation of ions such as copper and zinc, which are toxic to embryos.⁶

It is obvious from the literature that cleavage is possible to the blastocyst stage without serum, and that the amount of protein synthesis before the eight-cell stage is comparatively small but afterwards increases rapidly.⁷ The omission of serum could affect the embryos' metabolism at a later stage⁴ which is not detectable through the microscope.

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Epithelial ovarian tumours of low malignant potential

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Summary

The available literature and the management of epithelial tumours of low malignant potential (LMP) is reviewed. The criteria for a diagnosis of LMP at the University of the Witwatersrand are delineated in detail. Based on the records in the Ovarian Tumour Registry of this University, experience with 29 such tumours over 4 years is presented. Of these, 14 (48,3%) were of the serous variety, 12 were mucinous (41,4%), and 2 (6,9%) were mucinous-serous, the remaining 1 (3,4%) being endometrioid. LMP tumours accounted for 12,9% of proliferating epithelial ovarian tumours in black patients compared with 16,9% in white patients. Pelvipertoneal cytological washings for detection of malignant cells in patients with LMP tumours is mandatory.

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The traditional division of neoplasms into either malignant or benign groups is arbitrary as there are no fundamental differences in their behaviour except in degree.¹ Certain tumours exhibit some, but not all, features of malignant growth, while others may metastasize or implant and still maintain a non-aggressive growth pattern. This intermediate group of 'borderline' tumours has morphology and clinical behaviour confusing to both the pathologist and clinical oncologist.

It has been stated that 'accurate classification of ovarian epithelial neoplasms is the foundation of proper therapy'.² The ovarian epithelial tumours of low malignant potential (LMP) or borderline malignancy are a group that have only recently received attention in the literature.¹⁻³ Without recognition of a well-defined intermediate group of ovarian malignancy, reports on relative frequency, natural history and therapeutic response of ovarian carcinoma are variable. When an intermediate group was not categorized, the 5-year survival for stage I carcinoma of the ovary has been reported to be as high as 86%.² By excluding this group, the survival rate fell to 65%.² Likewise, if all stages of carcinoma are considered, the 5-year survival rate is of the order of 50-57% but if the intermediate or borderline tumours are excluded the rate falls to 35%.²

Exact criteria for inclusion in the LMP group remain in some instances unclear, as does the treatment. Both these aspects are addressed and an analysis of these lesions in the Ovarian Tumour Registry of the University of the Witwatersrand, Johannesburg, is recorded.

History and evolution of the concept. Taylor⁴ is credited with having first described in 1929 a group of hyperplastic papillary cystadenomas that could be confused with carcinoma. He noted that they occasionally produced multiple implantations on the peritoneum but none the less behaved predomi-

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