

Variability in different lots of commercial bovine serum albumin affects cell multiplication and hatching of rabbit blastocysts in culture

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Summary. Rabbit morulae were cultured *in vitro* for 4 days in a synthetic culture medium supplemented with two different lots of commercial bovine serum albumin (BSA) and two different amino acid formulations in a factorial 2×2 arrangement. One lot of BSA caused complete hatching of a proportion of blastocysts and formation of more than twice as many cells per blastocyst (hatched and unhatched) as that of the second BSA lot which did not cause complete hatching of any blastocysts. The mean cell numbers of hatched blastocysts were more than twice those of non-hatched blastocysts. There was no significant effect of amino acid formulation.

Introduction

In the presence of some samples of commercial bovine serum albumin (BSA) rabbit blastocysts in culture hatch from the zona (Kane, 1972; Maurer, 1978; Kane & Headon, 1980). In this laboratory we noticed that the blastocysts that hatch are those that appear to be growing most rapidly. To quantitate this effect the present study examined the relationship between blastocyst hatching and cell number in the presence of different lots of commercial BSA.

Materials and Methods

Embryos

New Zealand White does were induced to superovulate by treatment with FSH (twice daily s.c. injections for 3 days: 0.5 mg/injection) followed 1 day later by simultaneous injection of LH (0.5 mg, i.v.) and artificial insemination. FSH and LH were pituitary preparations from Armour, Kankakee, Illinois. Embryos at the early morula stage were collected from the oviduct 44-48 h after LH injection. Embryos recovered at this stage possess a thick mucin coat laid down by oviducal secretions. The flushing medium was a Hepes-buffered simple salt solution containing 0.1% defatted BSA (Kane, 1979). The embryos from 3-4 rabbits collected on one day were pooled and then washed free of oviduct fluid with repeated changes of the flushing medium. They were placed in culture in 0.5 ml droplets of culture medium under paraffin liquid (Merck, ART. 7162) in tissue culture dishes. Culture dishes were maintained at 38°C under a gas phase of 5% CO₂ in air. Embryos were cultured for 4 days and the numbers of non-hatched blastocysts and hatched blastocysts were recorded. Blastocysts were stained according to the method of Grayson (1978), first developed for mammalian oocytes. The method may be briefly described as follows.

Blastocysts in a small drop of medium were placed on a slide precoated with glycerine-albumin, then allowed to dry in air overnight. The air-dried slides were fixed in ethanol-acetic acid (2:1 v/v)

for 45 min, stained in aceto-orcein HCl for 30 min, then dehydrated successively in 50%, 60%, 80% and 100% ethanol, cleared in xylene and mounted in mounting medium under a cover slip. The number of cells per blastocyst was counted under high power with the aid of an eyepiece micrometer grid.

Culture medium. The basic culture medium contained 108 mM-NaCl, 4.78 mM-KCl, 1.71 mM-CaCl₂.2H₂O, 1.19 mM-KH₂PO₄, 1.19 mM-MgSO₄.7H₂O, 25 mM-NaHCO₃, 0.5 mM-sodium pyruvate, 1 mM-glucose, 100 units penicillin G/ml, 500 µg streptomycin sulphate/ml and the vitamins and trace elements of Hams F10 medium (Ham, 1963). This medium was used with the amino acids of F10 medium or of Menezo's B2 medium (Menezo, 1976).

Bovine serum albumin. Two different lot numbers (41F-9300 and 119C-9325) of crystallized BSA (Sigma London Ltd, Poole, Dorset, U.K.; Type A 4378) were used. The two lots of BSA were bought within a 6-month period and stored at -20°C.

Experimental design. The experiment was 2 × 2 factorial arrangement of the two lot numbers of BSA (41F-9300 and 119C-9325) and two types of amino acid mixture in a randomized complete block design. BSA was used at a concentration of 1.5%. Each block of the experiment consisted of the morulae collected on 1 day divided equally among the four treatments. The proportions of blastocysts and hatched blastocysts were analysed by orthogonally partitioned χ^2 tests with the Yates' correction for continuity. The blastocyst cell counts were analysed by least squares analysis of variance.

Results

One lot of BSA (No. 41F-9300) caused complete hatching of a proportion of the blastocysts formed whereas the second lot examined (No. 119C-9325) did not (Table 1). BSA No. 41F-9300 also stimulated a marked and significant increase ($P < 0.01$) in blastocyst cell number as compared with BSA No. 119C-9325 (Table 1).

There was no marked effect of type of amino acid formulation. There was some indication that the Medium B2 amino acids were superior when used with BSA No. 41F-9300 and the F10 amino acids with BSA No. 119C-9325. However, this interactive effect was not significant ($P > 0.05$).

Examination of the mean \pm s.e.m. cell counts of the hatched blastocysts (1168 \pm 83, 24 blastocysts) compared with those of the non-hatched blastocysts in treatments with BSA No. 41F-

Table 1. The effect of BSA lot and amino acid formulation on rabbit blastocyst formation, hatching and cell number in culture

	No. of morulae	% blastocysts	% hatched blastocysts	Blastocyst cell counts (mean \pm s.e.m.)
<i>Individual treatment means</i>				
BSA No. 41F-9300				
+F10 amino acids	51	96	41	710 \pm 50
+B2 amino acids	50	100	20	771 \pm 75
BSA No. 119C-9325				
+F10 amino acids	51	94	0	409 \pm 165
+B2 amino acids	48	100	0	297 \pm 166
<i>Overall means</i>				
BSA				
No. 41F-9300	101	98	31	742 \pm 46
No. 119C-9325	99	97	0	345 \pm 25
Amino acid formulations				
F10	102	95	21	598 \pm 39
B2	98	100	10	572 \pm 53

All percentages are based on the total no. of embryos in each treatment.

9300 (the only treatments in which complete hatching occurred) (549 ± 27 , 53 blastocysts) or the non-hatched blastocysts of all treatments (449 ± 21 , 104 blastocysts) showed that the mean cell counts of the hatched blastocysts were 2–3 times greater than for the non-hatched blastocysts ($P < 0.01$).

Discussion

Three points emerge from this experiment. Firstly, it documents the variability present in the effects of different samples of commercial BSA on embryos. This variability, while well known to workers using commercial BSA for embryo culture, is rarely documented in the literature. Quite clearly a medium containing BSA is not a defined medium.

Secondly, it shows that some samples of BSA stimulate cell division, blastocyst growth and blastocyst hatching. This mitogenic activity of some samples of BSA is potentially very important and present work in this laboratory indicates that it resides in a low molecular weight fraction which can be extracted from the BSA with 5% formic acid (M. T. Kane, unpublished data).

Thirdly, it shows that hatching of rabbit blastocysts in culture is associated with an increase in cell number. This fact, plus evidence that hatching of blastocysts in culture is not dependent on protease action (Kane, 1983), indicates that hatching of rabbit blastocysts *in vitro* is probably due to mechanical rupture of the zona and mucin coat (when one is present) by the growing blastocyst. *In vivo* the situation is clearly different in that blastocyst hatching does not occur. This is probably at least partly due to that fact that *in utero* the mucin coat laid down in the oviduct is added to by uterine secretion (Boving, 1957). It has also been shown that extensive remodelling of the blastocyst coverings takes place *in vivo* (Denker & Gerdes, 1979). There is evidence that *in-vitro* hatching is reduced by the presence of the mucin coat (Kane, 1975). Photomicrographs of the hatching process and of completely hatched blastocysts formed in the presence of BSA in culture have already been published (Kane, 1972, 1975; Maurer, 1978).

The use of BSA in embryo culture media for a number of species has caused great problems of variability of response between laboratories and even in the same laboratory over time. To take one example of considerable interest because of its potential relevance to work on cattle embryo transfers, the results of Tervit, Whittingham & Rowson (1972), who cultured cattle embryos in a simple medium supplemented with BSA, have rarely if ever been repeated. This present study indicates that even from the same supplier there may be a clear-cut variation between different lots of BSA in their effects on cell division and blastocyst hatching.

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