An investigation into the feasibility of culturing rat embryos in media derived from sheep serum

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Received 3 June 1993; revised 2 February 1994; accepted 10 August 1994

The use of three media to support growth and development in headfold Sprague-Dawley rat embryos cultured for 48 h, for possible use in a teratogen assay, was investigated using sheepserum (SM) and sheep serum mixed with rat serum (80% sheep:20% rat)(RSM). Rat serum (RM) was used as the control. The pCO_2, pO_2, pH, bicarbonate concentration, glucose content and osmolarity of the media were determined at 0 h, 24 h and 48 h after the start of incubation. The embryos were assessed at 24 h and 48 h using yolksac diameter, crown-rump length, somite number, protein content, stage of development and morphological scoring. Malformations were noted. The pCO_2 and pO_2 levels in the media were similar to those present in the gas environment. The RM had a lower pH ($P < 0.01$), less bicarbonate ($P < 0.01$) and a higher osmolarity ($P < 0.01$) than SM and RSM, which had similar levels, although RSM had a higher osmolarity ($P < 0.01$) than SM. A drop in pH and bicarbonate level was noted over the culture period. Glucose levels dropped only in media containing embryos. For all parameters measured, the embryos cultured in RM were superior to those cultured in RSM and SM, with the embryos in SM being inferior to those of RSM in certain aspects. The embryos in RSM had more malformations per embryo than those in RM and SM. The RM produced fewer abnormal embryos ($P < 0.01$) than SM and RSM, which were similar. Most abnormalities detected involved the neural tube. In general, the embryos in RSM were not much improved on those in SM. In conclusion, sheep serum could not support acceptable normal embryonic growth and development for a teratogen- detecting assay with the culture technique used in this study.

Die vermoë van media, vir moontlike bepaling van teratogeniese stowwe, om groei en ontwikkeling te onderhou van kopvou Sprague-Dawley rot-embrios gekweek vir 48 h, is ondersoek, deur gebruik te maak van skaapserum (SM) en skaapserum gemeng met rotserum (80% skaap-: 20% rotserum)(RSM). Rotserum (RM) is as kontrole gebruik. Die pCO_2, pO_2, pH, bikarbonaat konsentrasie, glukose inhoud en osmolariteit van die media is bepaal na 0 h, 24 h en 48 h kweking. Die embrios is by 24 h en 48 h geëvalueer vir dooiersakdeursnee, kroon- kruislengte, somietgetal, proteïeninhoud, stadium van ontwikkeling en morfologiese puntetoekenning. Misvormdhede is genoteer. Die pCO_2 en pO_2 vlakke was verteenwoordigend van dié in die gasatmosfeer. Die RM het 'n laer pH ($P < 0.01$), laer bikarbonaatvlakke ($P < 0.01$) en 'n hoër osmolariteit ($P < 0.01$) as SM en RSM getoon, wat soortgelyke vlakke gehandhaaf het, alhoewel RSM 'n hoër osmolariteit ($P < 0.01$) as SM gehad het. 'n Daling in pH en bikarbonaatvlakke oor die kwekingsperiode is opgemerk. Glukose konsentrasie het net gedaal in media wat embrios bevat het. Embrios gekweek in RM het beter vertoon teenoor RSM en SM ten opsigte van alle parameters gemeet, terwyl die embrios in SM swakker vertoon het teenoor dié in RSM wat sekere aspekte aanbetref. Die embrios in RSM het meer misvormings per embrio vertoon teenoor die in RM en SM. Die RM het minder abnormale embrios ($P < 0.01$) opgelewer as SM en RSM, wat soortgelyke resultate gelewer het. By die meeste abnormaliteite waargeneem, was die senubuis betrokke. Oor die algemeen het embrios in RSM nie beter gevaar as dié in SM nie. Gevolglik kan skaapserum nie aanvaarbare, normale embrionale groei en ontwikkeling vir 'n teratogeniese bepaling, met die kwekingstegniek gebruik in hierdie studie, handhaaf nie.

Keywords: Please supply.

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Introduction

The whole rat embryo culture technique has been used to detect teratogenic substances, reproductive disorders and reproduction-related nutritional disorders in serum of monkeys and humans (Klein *et al.*, 1982; Carey *et al.*, 1983; Chatot *et al.*, 1984; Gilles *et al.*, 1984; Ferrari *et al.*, 1986; Carey & Klein, 1989). Under South African conditions the lambing percentage of woolled breeds is only 74.3% (De Klerk *et al.*, 1983). The feasibility of using this culture technique as a simple assay prior to the breeding season to identify reproductive problems, so that corrective procedures can be adopted, was investigated.

Although rat embryos have been cultured successfully in short-term incubations in homologous (rat) serum (New, 1978), the success rate in sera from other species has been low. Little information on the use of sheep serum as a culture medium is available. New (1966a) found that sheep serum, not supplemented with glucose and not immediately centrifuged, was often harmful to rat embryos in culture, even with heat inactivation of the complement. Chatot *et al.* (1980) cultured rat embryos on glucose-supplemented, immediately-centrifuged, heat-inactivated, diluted (10%) human serum to the same stage of development seen with rat serum. Other authors have been unable to confirm these findings, although glucose supplementation did improve the growth and differentiation of rat embryos in human

(Reti *et al.*, 1982; Lear *et al.*, 1983; Priscott, 1983; Steele, 1985) and dog (Steele & Marlow, 1985) serum. Normal development of rat embryos cultured on glucose-supplemented monkey serum has been reported (Klein *et al.*,1982). Priscott (1983) cultured 10.5-d rat embryos for 48 h in sheep serum. This serum proved to be inferior to rat serum in supporting embryonic growth and differentiation. However, the medium used to dilute the sheep serum was reported to be toxic to early embryos when used on its own (Sadler & Warner, 1984).

Rat embryos cultured in heterologous sera do not develop a circulatory system and are anaemic. The addition of 10 – 25% rat serum corrected this and also improved the growth and development in human, dog and pig serum (Reti *et al.*,1982; Lear *et al.*, 1983; Priscott, 1983; Steele & Marlow, 1985). Embryonic growth and development was, however, inferior to rat serum on its own.

There is a paucity of literature available on changes occurring in the media during 48-h rat embryo culture. Embryos are very sensitive to changes in the culture medium environment and will, as a result, develop abnormally when conditions are less than optimal. Little is known about the influence of culture environment on the medium parameters of pH, pCO_2, pO_2, bicarbonate levels, glucose content and osmolarity during 48-h culture. Osmolality is important for embryonic viability, growth and development (Collins & Hallett, 1983) and it has been shown that raised osmolarity will increase the frequency and severity of malformations under teratogenic conditions (Cockcroft & Coppola, 1977). No evidence exists that stabilization of factors such as pH, pCO_2, pO_2 and osmolarity would significantly improve growth and development in cultured rat embryos (Priscott, 1979). However, New & Cockcroft (1978) suggested that the pH, pCO_2 and pO_2 may be critical in rat embryo culture and that conditions under which embryos are grown should be defined.

Since only one report was available on glucose-supplemented sheep serum, the growth supporting capabilities of this serum needed to be determined prior to establishing an assay. This study (a) investigated whether 9.5-d rat embryos could be cultured successfully on glucose-supplemented sheep serum and, if not, whether an improvement could be seen with the addition of rat serum, and (b) determined pH, pCO_2, pO_2, bicarbonate levels, glucose content and osmolarity of the medium during culture. Rat serum was to serve as a control. Some of these results were reported previously in preliminary form (Burgman *et al.*, 1990).

Materials and Methods

Animals and blood sampling

Twenty multiparous Merino-type ewes were sampled from the vena jugularis four days after oestrus to minimize any hormonal influences. Sprague-Dawley female rats were used for terminal blood sampling via an abdominal aorta catheter and as embryo donors (noon of the day on which a vaginal plug was found was regarded as day 0.5 of gestation).

Culture media preparation

The blood was prepared according to the methods of Steele & New (1974). Serum which showed signs of haemolysis was discarded. A combination serum containing 80% sheep serum and 20% rat serum was prepared. All media were prepared simultaneously. The serum was heat-inactivated at 56°C for 30 min. The medium for culture consisted of 90% serum (v/v), supplemented with glucose (final concentration between 200 and 300 mg/100 ml), antibiotics and water up to 100% (Chatot *et al.*,1980), and filtered (0.20 µm). The medium (2 ml) was immediately placed into sterile polypropylene culture tubes (Falcon), capped and stored at –20°C.

Explantation procedure

Between 12:00 and 14:00 on day 9.5, the pregnant animals were killed by cervical dislocation. The uteri were aseptically resected and placed in Earle's buffered solution (Highveld Biological, Republic of South Africa). The embryos were removed according to New (1966b). Prior to culture, each embryo was evaluated for intact membranes and only those which were intact were used.

Culture procedure

All selected embryos were pooled before being placed randomly into the culture. The embryos were then transferred into the culture tubes (1 ml/embryo; 2 embryos/tube; New, 1966a). The tubes were capped loosely and placed onto a rotating drum (58 rpm) inside the incubator at 37°C (New *et al.*, 1973). The gassing regime of Steele (1985) was used.

Embryo assessment

The embryos were assessed at 24 h and 48 h, according to the morphological scoring system of Brown & Fabro (1981). Other parameters, such as yolk sac diameter (YSD), crown-rump length (CRL) and somite count, were also determined. For protein content, the embryos were dissolved in 1 ml of 1N NaOH and analysed using the method of Lowry *et al.*, (1951), as modified by Keleti & Lederer (1974).

Media assessment

Osmolarity was determined by freezing point depression using a Knauer (West Germany) micro-osmometer. The pO_2, pCO_2, pH and bicarbonate were determined on a Radiometer (Denmark) ABL 1 Acid-Base analyzer. Glucose content was determined using a GOD-PERID kit (Boehringer – Mannheim). Media were sampled at 0 h, 24 h and 48 h. Control samples (no embryos) were incubated simultaneously.

Statistical analysis

Analysis of variance and Scheffe's multiple range test were used on all parameters with the exception of the malformations. The malformations were analysed using the Chi-squared test and the log-linear analysis of Goodman (Steyn *et al.*, 1987).

Results

Embryonic growth and development

The data are summarized in Table 1 for embryos after 24 h and 48 h in culture. Although the rat medium (RM) produced significantly larger yolk sacs at 24 h, after 48 h no significant difference could be detected between the three media. With the aid of regression curves, various growth rates were determined relative to those embryos in RM. Using CRL, the growth rate of embryos in the combined medium (RSM) was 51.9% and in the sheep medium (SM) 29%. The rate of somite development was 67.6%

Table 1 Physical parameters (mean standard deviation) obtained for the rat embryos cultured for 24 h and 48 h

Data	Rat medium	Rat:Sheep medium	Sheep medium
24-h culture			
n	50	48	50
YSD (μm)	2076.31 ± 296.46[a]	1791.46 ± 321.86[b]	1792.98 ± 259.11[b]
CRL (μm)	1639.61 ± 334.12[a]	1218.70 ± 197.57[b]	1133.83 ± 196.32[c]
SOM (n)	11.02 ± 1.66[a]	9.60 ± 1.93[b]	8.74 ± 1.99[c]
MORPH.SC.	15.01 ± 2.99[a]	9.65 ± 1.67[b]	7.76 ± 1.63[c]
PROTEIN (μg)	13.72 ± 3.11[a]	8.04 ± 1.96[b]	6.86 ± 1.44[c]
48-h culture			
n	49	50	50
YSD (μm)	2960.31 ± 668.66[a]	2901.07 ± 543.13[a]	2806.00 ± 418.72[a]
CRL (μm)	2636.28 ± 410.57[a]	1735.68 ± 333.51[b]	1418.88 ± 360.29[c]
SOM (n)	17.88 ± 4.82[a]	13.3 ± 3.53[b]	11.04 ± 3.11[c]
MORPH.SC.	31.90 ± 5.48[a]	16.23 ± 4.51[b]	11.57 ± 3.97[c]
PROTEIN (μg)	45.79 ± 18.42[a]	19.44 ± 7.33[b]	11.76 ± 4.85[c]

YSD = yolk sac diameter; CRL = crown-rump length; SOM = somite count; MORPH.SC. = morphological score

[a,b,c] $P < 0.01$ Rows with the same superscript do not differ significantly from each other

in RSM and 52.9% in SM. The rate of protein accumulation was 35.1% in RSM and 14.9% in SM.

The embryonic development attained in each medium is shown in Table 2 for the 24-h culture period, and in Table 3 for the 48-h culture period. Relative to embryos in RM, at 24 h the embryos cultured in RSM and SM were retarded in development and at 48 h the difference was even larger. In general, RSM did not fare much better than SM.

At 24 h a significant difference ($P < 0.01$) was noted in the number of abnormal embryos in the three media. The RSM had significantly ($P < 0.01$) more embryos which showed malformations than SM. At 48 h there was a sharp increase in the number of embryos showing malformations. The RM had significantly fewer ($P < 0.01$) abnormal embryos than the other media, which did not differ from each other. The medium had a significant ($P < 0.01$) influence on the number of malformations per embryo. At 24 h one abnormality at most per embryo was seen. At 48 h, however, the number of abnormalities per embryo increased to a maximum of four. At 48 h, 61% of the embryos cultured in SM had one abnormality, which differed significantly ($P < 0.01$) from the other media. Embryos in RM had significantly ($P < 0.01$) more embryos with no abnormality than the other media. The malformations observed are given in Table 4.

The most common abnormality observed at 24 h was the presence of haemorrhaging in the midbrain area (85.71%). At 48 h, the fusion of the anterior neural folds to the posterior neural

Table 2 Embryonic development after the 24-h culture. Percentages are in brackets

Medium	Total embryo numbers	Corona Blood Islands (yolk sac)	Turning	Anterior neuropore formed	Optic primordium	One branchial bar	Fusion neural tube at level 4/5 somites
Rat	50	35 (70)[a]	12 (24)[a]	29 (58)[a]	42 (84)[a]	31 (62)[a]	48 (96)[a]
Rat:sheep	48	32 (67)[a]	1 (2)[b]	0 (0)[b]	2 (4)[b]	3 (6)[b]	3 (6)[b]
Sheep	50	2 (4)[b]	0 (0)[b]	0 (0)[b]	3 (6)[b]	2 (4)[b]	2 (4)[b]

Columns with the same superscript do not differ significantly from each other

[a,b,c] $P < 0.01$

Table 3 Embryonic development after the 48-h culture. Percentages are in brackets

Medium	Total embryo numbers	Yolk sac circulation	Turning complete	Anterior neuropore closed	Optic vesicle	Otocyst	Two or more branchial bars	Fusion of allantois	Fore-limb buds	Posterior neuropore open	Closure neural tube
Rat	49	38 (78)[a]	29 (59)[a]	42 (86)[a]	42 (86)[a]	30 (61)[a]	44 (90)[a]	45 (92)[a]	24 (49)[a]	27 (55)[a]	22 (45)[ad]
Rat:sheep	50	2 (4)[b]	1 (2)[b]	0 (0)[b]	2 (4)[b]	7 (14)[b]	16 (32)[b]	47 (94)[a]	1 (2)[b]	4 (8)[b]	6 (12)[be]
Sheep	50	0 (0)[b]	1 (2)[b]	2 (4)[b]	1 (4)[b]	3 (6)[b]	2 (4)[c]	46 (92)[a]	0 (0)[b]	3 (6)[b]	0 (0)[bf]

Columns with the same superscript do not differ significantly from each other

[a,b,c] $P < 0.01$

[d,e,f] $P < 0.025$

Table 4 Abnormality type detected in each culture medium at 24 h and 48 h

Medium	Culture period	*n*	Abnormal midbrain folds	Haemorrhaging present in:			Abnormal neural tube fusion ('Squirrel')	Abnormal fore-brain and optic development	Other	Total
				Midbrain	Fore-brain	Neural tube				
Rat	24	50	0	3	0	0	0	1	0	4
	48	49	0	0	2	0	0	2	1	5
Rat:sheep	24	48	0	9	0	0	0	1	0	10
	48	50	6	25	2	6	33	12	1	85
Sheep	24	50	0	0	0	0	0	0	0	0
	48	50	2	6	1	1	39	7	0	56
Total		297	8	43	5	7	72	23	2	160

folds ('squirrel' embryos) accounted for 49% of the abnormalities seen. Ninety-eight per cent of all the abnormalities detected involved the neural tube.

Media parameters

The results on media sampled at 0 h, 24 h and 48 h are given in Table 5. The pO_2 followed the oxygen concentration of the gas phase. At 48 h, the media containing embryos had a lower pO_2 than the control ($P < 0.01$). The pCO_2 increased from 12 mm Hg at 0 h to 24 mm Hg at 24 h ($P < 0.01$), and then remained constant.

The RM had a lower pH (7.884) and less bicarbonate (23.20 mmol/l) initially ($P < 0.01$) than the RSM (7.989 and 28.74 mmol/l) and SM (7.984 and 30.19 mmol/l) and this wis maintained throughout the culture period. The pH and bicarbonate concentration decreased ($P < 0.01$) from initial values at 24-h culture in the control and the treated medium, and at 48 h, only the treated medium showed a further decline.

No change in glucose levels occurred in the control medium, but a significant decline ($P < 0.01$) occurred in all media containing embryos. The mean initial osmolarities of SM (272 mosmol) and RSM (277 mosmol) were significantly lower ($P < 0.01$) than RM (284 mosmol). The SM had a lower ($P < 0.01$) osmolarity than the RSM. The osmolarity increased ($P < 0.01$) with time.

Discussion

The rate of growth and development of embryos cultured in rat serum were slower than those obtained by other laboratories (Steele & New, 1974; New *et al.*, 1976a; 1976b; Cockcroft & Coppola, 1977; Cockcroft, 1979; Klein *et al.*, 1980; Lear *et al.*, 1983; Lewandowski *et al.*, 1985; Steele, 1985). The SM produced retarded, small, anaemic and abnormal embryos and these results agree with the results of New (1966b), although the latter study did not use medium supplemented with glucose and serum obtained from blood which had been centrifuged on withdrawal, showed to be advantageous by Steele & New (1974). In this study, RSM improved growth parameters such as protein content, somite development, crown-rump length and embryonic scoring compared with embryos in SM, but was not comparable with embryos in RM. The anaemia was corrected, as reported previously (Reti *et al.*, 1982; Lear *et al.*, 1983; Huxham & Beck, 1984; Steele & Marlow, 1985). The embryonic development exceeded that in SM in the corona of blood islands, neural tube closure and development of two or more branchial bars. These

Table 5 Data (mean ±s.d.) obtained for the parameters pO_2, pCO_2, pH, bicarbonate level (HCO_3^+), glucose content and osmolarity measured in three media at the different culture periods

	Rat						Rat:sheep						Sheep					
	0 h		24 h		48 h		0 h		24 h		48 h		0 h		24 h		48 h	
Data	Cont	Trtd	Cont	Trtd	Cont	Trtd	Cont	Trtd	Cont	Trtd	Cont	Trtd	Cont	Trtd	Cont	Trtd	Cont	Trtd
n	11	22	13	25	12	25	11	25	13	25	12	25	11	25	13	25	12	25
pO_2	114.05	109.89	83.48	80.61	181.99	157.30	126.72	120.43	86.35	84.60	181.:37	162.00	130.67	128.57	86.20	88.95	177.35	164.19
mm Hg	±22.99	±21.68	±21.03	±18.77	±18.02	±12.01	±15.40	±16.63	±23.72	±24.96	±11.86	±16.82	±16.85	±15.61	±21.82	±20.66	±19.23	±13.18
pCO_2	12.94	12.18	24.92	24.66	25.38	24.92	12.40	12.00	24.10	24.09	24.81	25.18	12.07	12.30	24.37	23.99	24.85	24.27
mm Hg	±3.69	±3.99	±6.16	±6.67	±3.31	±3.44	±4.95	±4.01	±5.81	±6.60	±2.34	±3.49	±3.75	±3.15	±6.41	±6.69	±2.48	±3.45
pH	7.874	7.889	7.377	7.272	7.378	6.952	7.973	7.997	7.558	7.487	7.506	7.299	7.972	7.989	7.555	7.504	7.527	7.404
	±0.061	±0.056	±0.138	±0.155	±0.058	±0.218	±0.068	±0.057	±0.170	±0.162	±0.049	±0.106	±0.058	±0.055	±0.165	±0.196	±0.049	±0.094
HCO_3^+	24.18	22.73	15.39	11.10	14.64	5.64	29.19	28.52	22.12	17.69	19.48	12.04	29.33	30.58	20.00	18.98	20.54	14.95
mmol/l	±4.10	±4.04	±2.18	±1.37	±1.60	±1.16	±6.65	±3.79	±5.83	±2.38	±1.47	±1.76	±5.39	±4.63	±4.72	±2.18	±1.75	±1.66
Glucose	232.22	242.09	232.71	186.84	240.58	113.30	259.77	269.39	245.84	217.23	254.59	168.82	265.75	266.71	276.21	232.12	266.77	184.30
mg/100 ml	±15.27	±21.11	±18.36	±13.42	±16.24	±14.17	±24.38	±19.81	±13.98	±15.84	±19.35	±13.92	±26.03	±25.87	±44.08	±21.89	±14.88	±16.14
Osmol.	282.34	284.87	290.59	289.24	288.62	294.28	275.30	279.32	284.55	283.92	286.89	285.99	271.54	272.33	280.84	279.29	283.25	282.28
mosmol	±7.55	± 9.49	±8.43	±9.70	±13.43	±11.95	±13.69	±8:65	±6.95	±4.32	±8.81	±7.57	±8.99	±7.28	±5.85	±5.87	±3.98	±8.35

Cont = control; Trtd = treated; Osmol. = osmolarity; *n* = no. of independent cultures

embryos were inferior to those with values reported in the literature for heterologous sera.

The incidence of abnormalities in RM was approximately 8%, which was reported by Cockcroft & New (1975). The occurrence of abnormalities in RSM and SM was higher than values reported in the literature for other media. Most abnormalities involved the neural tube, which has been reported for human (Steele, 1985; Steele & Marlow, 1985) and dog (Steel & Marlow, 1985) sera. As reported for human serum (Chatot *et al.*, 1980), the abnormal embryos had less protein, incomplete body curvature and neural tubes that failed to close normally. Abnormal closure occurs when oxygen levels are above 10% during fusion (New *et al.*, 1976a; Morriss & New, 1979). At 24 h, the oxygen concentration increases to 20% and, as the development of embryos in RSM and SM is retarded, it affects neural tube fusion. The somites of the embryos in the mixed and sheep serum were ill-defined, which has been reported under adverse conditions (Cockcroft & New, 1975).

Due to the low pCO_2 (as a result of an altitude of 1523 m above sea level), the buffering capability of the media was affected (25 mm Hg in this study versus 35 – 48 mm Hg under normal physiological conditions (Meyer, 1983)). The retarded embryonic growth in the media and increased number of abnormalities seen per embryo in RSM could be due to the unphysiological pH.

The differences in the ability of different media to support normal rat embryo development *in vitro* appear to be related to the protein constituents of the serum (Rhinehardt *et al.*, 1984). Inefficient uptake and processing of essential macromolecules by the yolk sac may play a role in the inferior embryonic growth and development seen in heterologous media (Priscott, 1983). The inefficient selective uptake of bulk human serum proteins by the yolk sac was improved by the addition of 10% rat serum, producing normal, well-developed embryos (Huxham & Beck, 1984). Differences in protein composition were detected between the media (Burgman, 1990; Burgman & Morgenthal, 1994).

The dilution of RM with fluids, such as water and saline, has been found to retard embryonic growth (New, 1973; Priscott, 1983). Although Klein *et al.* (1980) found that the dilution of serum reduced the frequency of 'squirrel'-type embryos, this was not the case in this study. The 10% serum dilution may be harmful as a change in the osmotic relationship between the culture medium and the yolk sac can occur and affect the transfer of nutrient substances to the embryo and could reduce the rate of protein uptake by the visceral endoderm (New, 1973). The inferior growth seen in heterologous media could be due to an associated physical property, such as osmotic pressure (which may be different in rat serum), resulting in altered yolk sac function (Priscott, 1983). The possibility of an antigenic reaction occurring between the rat and sheep serum in RSM cannot be ignored. The presence of anti-species cell aglutinins in heterologous sera could exert toxic effects on the yolk sac or embryo and would persist even if sufficient rat serum were added to produce adequate embryonic development (Priscott, 1983). Immunoglobulins acting on the yolk sac or embryo cause growth retardation and abnormalities (Carey & Klein, 1989). The excessive haemorrhaging observed in the embryos cultured in RSM implies that haemoglobin formation and development of the circulatory system were disrupted and could be an example of this.

Embryos cultured in bovine serum for 48 h had exencephaly, anophthalmia and abnormal body curvature. The addition of the amino acid methionine to the culture medium overcame these defects. It must be mentioned that changing the gassing regime also had the same effect (Klug *et al.,* 1985). Normal bovine serum contains 22.7 nmoles/ml methionine compared with 61.7 nmoles/ml present in rat serum. Increasing this level in cattle serum allowed normal development to take place (Dealy *et al.,* 1986). Since sheep are ruminants, the possibility exists that endogenous blood methionine levels could be lower than those in rat serum. This could explain the retarded abnormal development seen in the media containing sheep serum. Anophthalmia was not seen in this study.

In conclusion, embryonic growth and development in RSM and SM are not comparable to that seen in RM and insufficient for the establishment of an assay in its current form. The addition of rat serum to sheep serum still produced inferior embryos, although an improvement on those cultured in sheep serum alone was seen in some parameters and the anaemia was corrected. This improvement was negated by the high incidence of embryonic abnormalities thus making this medium an unacceptable growth medium for rat embryos.

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