INTRA- AND INTER-SPECIFIC TRANSFER OF OVA BETWEEN EXPLANTED RAT AND MOUSE OVIDUCTS

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Summary. Intra- and inter-specific transfers of rat and mouse ova showed that native and foreign ova developed to the blastocyst stage within explanted oviducts of the rat and mouse.

Reports in the literature indicate that ova can develop in foreign oviductal environments *in vivo* at least to the blastocyst stage. Warwick & Berry (1949) reciprocally transferred the cleavage stages of sheep and goats and obtained blastocysts in both instances. Averill, Adams & Rowson (1955) found that the cleavage stages of the sheep developed to 8-day blastocysts when transferred to the rabbit oviduct. Briones & Beatty (1954) were unsuccessful in their attempts to transfer cleavage stages between rat, mouse, guinea-pig and rabbit. However, Tarkowski (1962) reciprocally transferred the cleavage stages of the rat and mouse and obtained development to the blastocyst stage.

Reciprocal and non-reciprocal transfer of ova to explanted ampullae of the rat and mouse have been investigated to determine whether: (1) rat ova will develop in organ culture since they are somewhat refractory to the *in vitro* methods of culture used so successfully in the mouse (Brinster, 1963); and (2) the oviductal environment of the explanted ampullae from either species will support growth of foreign ova.

Methods of preparation and culture of mouse oviducts and the collection of zygotes have been described elsewhere (Whittingham, 1967, 1968a). Immature female albino rats, 26 to 28 days old, were injected intraperitoneally with 30 i.u. of PMSG followed 48 hr later by 10 i.u. of HCG. After the second injection, the vagina was opened and the rats placed with proven males. The following morning they were checked for mating by examining the vaginal smears for spermatozoa. Fertilized 1-cell ova were obtained from successfully mated females and ampullae were removed from unmated animals, flushed with culture medium (Whittingham, 1968a) and placed in organ culture. The ampullar region of the oviduct was cultured since it has been shown previously that this region, but not the isthmus, supports development of mouse zygotes in organ culture (Whittingham, 1968a).

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In the first series of experiments rat and mouse zygotes were reciprocally and non-reciprocally transferred to explanted ampullae (twelve ova/ampulla). Organ cultures containing mouse zygotes were cultured for 96 hr and those containing rat ova for 120 hr since rat zygotes reached the blastocyst stage *in vivo* approximately 24 hr later than mouse zygotes. The results are summarized in Table 1. The data were subjected to χ^2 analyses using Yates correction for continuity. The intra-specific transfer of mouse zygotes was significantly different from the other three combinations ($P < 0.001$). Comparisons of the differences between the combinations of rat ova to mouse ampulla and mouse ampulla to rat ampulla were not significant. The combination of rat ova into rat ampullae was not very successful, only five ova from 141 ova recovered had reached the morula stage. The lower response in the number of mouse ova developing in the rat ampulla (31.0% blastocysts and morulae) may be due either to the foreign environment of the rat ampulla or to the poor maintenance of the rat ampulla in organ culture. The rat ampullae after 4 to 5 days in culture

TABLE 1

DEVELOPMENT OF INTRA- AND INTER-SPECIFIC ZYGOTES WITHIN EXPLANTED AMPULLAE FROM THE RAT AND MOUSE

Type of transfer	No. ampullae cultured	Duration of culture (hr)	No. ova recovered/ No. ova transferred	No. morulae	No. blastocysts	% Morulae and blastocysts
Mouse → Mouse	10	96	114/120	11	53	56.1
Rat → Rat	16	120	141/192	5	0	3.5
Mouse → Rat	10	96	100/120	13	18	31.0
Rat → Mouse	22	120	245/264	11	45	22.1

on a chemically defined medium (Ham's nutrient mixture F10; Ham, 1963) were very friable and starting to disintegrate. This was in contrast to the apparently healthy appearance of the mouse oviduct after 4 days in culture (Whittingham, 1967), although the mouse ampulla did become more fragile after 5 days in culture. Nevertheless the overall results demonstrate that both rat and mouse ova will develop in explanted ampullae of either species in organ culture.

A second series of experiments was designed to determine if 2-cell rat ova would develop better in organ culture than the zygote. Two-cell rat ova (twelve/ampulla) were transferred to ampullae of the rat or mouse explanted at Metoestrus I (Whittingham, 1968b). The ampullae were cultured for 96 hr and then flushed. The results are presented in Table 2. The development of a few 2-cell rat ova (19% blastocysts and morulae) within the rat ampulla suggests that the shorter period of culture is less detrimental to the ovum. Perhaps 2-cell rat ova are less susceptible to adverse changes in the oviductal environment arising from manipulation and organ culture. The development of 2-cell rat ova within the mouse ampullae (46.8% blastocysts and morulae) would support this view.

This is the first recorded report of the successful development of 1- and 2-cell rat ova to the blastocyst stage obtained by *in vitro* organ culture techniques used successfully to culture the mouse zygote (Biggers, Gwatkin & Brinster, 1962; Whittingham, 1968a). Earlier attempts to cultivate the rat cleavage stages in a completely *in vitro* system have had limited success (Mark & Long, 1912; Defrise, 1933; Washburn, 1951; Wrba, 1956).

Rat ova developed within explanted ampullae maintained on a completely chemically defined medium (Ham's F10). This medium did not support the early cleavage stages outside the oviduct. Therefore the ampulla region of the oviduct must have provided the essential nutritive and protective requirements for the developing rat embryo. The poor maintenance of the rat oviduct in culture may be improved by the addition of the serum to the medium and warrants further investigation.

TABLE 2

DEVELOPMENT OF 2-CELL RAT OVA AFTER TRANSFER TO EXPLANTED RAT AND MOUSE AMPULLAE

Type of transfer	No. ampullae cultured	No. ova recovered/ No. ova transferred	No. morulae	No. blastocysts	% Morulae and blastocysts
Rat → Rat	6	63/72	10	2	19.0
Rat → Mouse	11	111/132	14	38	46.8

* Ampullae cultured for 96 hr.

The reciprocal transfers confirmed, in part, the *in vivo* findings of Tarkowski (1962). However, he did not transfer ova at the 1-cell stage but showed that later cleavage stages (2-cell, 4-cell and 8-cell ova) would develop to the blastocyst stage in the foreign oviduct.

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