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The effects of oestradiol-17β and tamoxifen on the development of mouse embryos cultured over collagen

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The late preimplantation mouse blastocyst becomes noticeably sticky after its escape from the zona pellucida. Around this time there is a change in the staining reaction of its trophoblastic surface coat to colloidal iron-Prussian blue. Whereas the surface coat of the 80–86 h *post-coitum* (h p.c.) blastocyst stains red that of the 96–100 h p.c. blastocyst, i.e. after the surface coat change (SCC), stains blue. Implantation in the mouse is an oestrogen-dependent phenomenon and so is the surface coat change (Holmes & Dickson, 1973). Tamoxifen, a non-steroidal antioestrogen and a drug which prevents implantation in the mouse, prevents the SCC *in vivo* (Bloxham, Pugh & Sharma, 1975). It is also known that blastocysts can be induced to undergo the SCC *in vitro* if to the Whittingham's (1971) medium in which they are incubated is added oestradiol-17β at a concentration greater than 1.5×10^{-10} M and that this effect of oestradiol-17β is antagonized by tamoxifen in a concentration dependent manner (Bloxham & Pugh, 1977).

The importance of oestradiol to maturation and implantation had been called into doubt, however, when Jenkinson & Wilson (1973) showed that mouse blastocysts in Whittingham's medium would develop normally and even go on to mimic the phases of attachment and invasion if incubated over a layer of reconstituted collagen. It was therefore of interest to investigate 80 h p.c. embryos grown in the Jenkinson & Wilson system (groups of 19–26 embryos in 2 ml Whittingham's medium) for the occurrence of the SCC.

The change had occurred in all 8 morphologically normal embryos removed at 36 h of culture and by 68 h all morphologically normal (24 out of 32) embryos had attached to the collagen. In the absence of collagen all embryos remained free-floating and none of the 15 stained at 16 h or 36 h had undergone the SCC. In the absence of collagen but presence of oestradiol (10^{-8} M), all 9 blastocysts examined had undergone the SCC after 16 h but none of the 14 remaining became attached.

The addition of oestradiol (10^{-8} M) to the collagen containing culture system allowed the percentage of blastocysts which attached to rise from 74.28% to 90.47% ($P < 0.05$) and attachment occurred 10 h earlier. The further addition of tamoxifen (2.8×10^{-10} M) caused the proportion of blastocysts which became attached to fall to 39.13% at 68 h of culture. In the absence of oestradiol, SCC and implantation were totally prevented by tamoxifen (2.8×10^{-10} M).

It can be concluded that the simultaneous presence of oestrogen and an appropriate surface increase the rate of development and the frequency of attachment of mouse embryos in culture, that the anti-implantation action of tamoxifen *in vitro* is independent of exogenous oestradiol and that the surface coat change precedes implantation *in vitro*.

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