Effect of an anti-oestrogen on implantation of mouse blastocysts

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Summary. Mouse blastocysts collected on Day 4 were cultured for 4 h in Whitten's medium containing 5 µg CI-628 citrate, a specific oestrogen antagonist, and transferred to the uteri of pseudopregnant hosts. There was a significant reduction in the number of implantations observed on Day 11 when compared with those from blastocysts which were similarly transferred after culture in medium alone or in medium containing 1 µg oestradiol-17β and 5 µg CI-628 citrate. We suggest that this is further evidence for the involvement of embryonic oestrogen in implantation.

Introduction

Implantation involves an initial attachment of the blastocyst to the surface of luminal epithelium and this cellular contact could then allow an exchange of information from the blastocyst to epithelial cells. Although the nature of such a signal is still unknown, it is thought to be a humoral substance released by an implanting blastocyst (Heald, 1976). It has been proposed that steroids synthesized or accumulated in the blastocyst may play an important role in implantation (Dickmann, Dey & Sengupta, 1976; George & Wilson, 1978) and in triggering the local cellular events that occur at the site of implantation (Sengupta, Roy & Manchanda, 1979). The present study was designed to test whether embryonic oestrogen is essential for implantation in the mouse by exposing blastocysts directly to an anti-oestrogen.

Materials and Methods

Random-bred Swiss mice were killed on Day 4 of gestation (Day 1 = day of finding copulatory plug) to recover blastocysts. After a brief wash in culture medium, the embryos were randomly assigned to one of three groups. In Group I the blastocysts were placed in glass tubes and cultured for 4 h in Whitten's culture medium supplemented with 0.3% crystalline bovine serum albumin (Sigma Co.) according to the method described by Whitten (1971). In Group II, blastocysts were similarly cultured for 4 h in medium containing 5 µg CI-628 citrate (Parke Davis Co., U.S.A.). In Group III, the culture medium contained 5 µg CI-628 citrate and 1 µg oestradiol-17β; blastocysts were cultured for 4 h. At the end of culture, the blastocysts from all 3 groups were rapidly examined under a stereomicroscope, washed in fresh culture medium under sterile conditions and then carefully transferred to the uteri of females on Day 3 of pseudopregnancy. An average of 7 or 8 blastocysts were transferred to each uterine horn. Pseudopregnancy in the host mice was induced by removing both oviducts on Day 1 of pregnancy. The host animals were killed on Day 11 and the number of implantations as well as fetal and placental weights were carefully recorded. A fetus was considered to be live and viable if it showed a fast-beating heart. A group of 7 normal pregnant mice (Group IV) was left

untreated as control and these were also killed on Day 11 to assess the range of normal fetal and placental weights.

Results

As shown in Table 1, the percentages of live fetuses in Groups I and III were similar, but the treatment in Group II resulted in very few fetuses. Fetuses in all 3 groups were viable as observed from their heart beats. Placental weights differed little in the 4 groups; the fetuses in Group II were significantly heavier.

Table 1. Effect of exposure of Day-4 mouse blastocysts to an anti-oestrogen before transfer to the uteri of pseudopregnant hosts

Group	No. of blastocysts transferred (no. of recipient uterine horns)	Total no. of viable fetuses (%)	% of females with viable fetuses	Fetal wt (mg)	Placental wt (mg)
I	130 (17)	52 (40)*	58.8	14.25 ± 0.08‡	28.31 ± 1.05
II	128 (17)	21 (16.4)*†	35.3	28.94 ± 2.67‡	29.74 ± 5.61
III	114 (15)	42 (36.8)†	73.3	12.55 ± 0.58	27.47 ± 1.29
IV	—	—	—	13.26 ± 0.74	27.91 ± 1.95

Values are mean ± s.e.m.

*† Significantly different, $P < 0.001$ (proportion, Z. test).

‡ Significantly different, $P < 0.001$ (t test).

Discussion

The hormonal requirements for implantation have been known for a long time but the precise mechanisms which trigger embryo attachment and implantation remain to be understood. Blastocysts obtained from a number of species have been shown to contain two key steroidogenic enzymes and also steroids such as progesterone, oestradiol-17 β and their metabolites (Fuchs & Beling, 1974; Dickmann *et al.*, 1976; George & Wilson, 1978). In ovariectomized and progesterone-treated rabbits implantation is dependent upon embryonic oestrogen (Bhatt & Bullock, 1974; Dey, Dickmann & Sengupta, 1976; George & Wilson, 1978), but this mechanism may not be applicable to mice and other rodents.

McCormack & Greenwald (1974) and Sengupta *et al.* (1979) have shown that in mice ovariectomized at 15:00 h on Day 4, normal implantations occur. However, when such ovariectomized animals are injected intraperitoneally with an oestrogen antagonist such as CI-628 citrate, implantation failure was shown by the absence of characteristic blue dye sites and local cellular lysosomal enzymic changes in uterine epithelial cells surrounding the blastocyst (Sengupta *et al.*, 1979). We therefore suggested that in addition to the systemic oestrogen source which supplies the steroid throughout the uterine horn, there may be a further need for local oestrogen to initiate the vascular response and other cellular events during the early stages of embryo attachment. In the experiments of Sengupta *et al.* (1979) the drug was given by intraperitoneal injection and so the present experiment was designed to expose the blastocysts directly. The finding that exposure of blastocysts to the anti-oestrogen significantly inhibited implantation is compatible with the hypothesis that blastocyst oestrogen could participate in inducing local cellular and vascular changes at implantation. Logeat, Sartor, Hai & Milgrom (1980) have shown a local effect of the blastocysts on oestrogen and progesterone receptors in the rat endometrium. Nuclear receptors for both steroids were found in 2-fold higher concentration in implantation sites than in non-implantation of regions of the endometrium of

6-day pregnant rats. Moreover, this difference in receptor concentration was not observed in decidual tissue obtained from pseudopregnant animals. In mice and rats, implantation sites are associated with low uptake of exogenously administered [^3H]oestradiol-17 β in comparison with non-implantation uterine areas, suggestive of local saturation of oestrogen receptor sites with the steroid released from the blastocyst (Sartor, 1977; Ward, Frost & Orsini, 1978). All these observations suggest that there is involvement of oestrogen at the site of implantation. On the other hand, rabbit blastocysts have the capacity to accumulate oestrogen and progesterone from the surrounding uterine fluid (Borland, Erickson & Ducibella, 1977; Angle & Mead, 1979) and also metabolize progesterone and androgens (Huff & Eik-Nes, 1966; Singh & Booth, 1978). Whether of embryonic origin or from a maternal source, oestrogen is likely to be present in blastocysts at the time of implantation.

Callantine *et al.* (1966) considered that CI-628 exerts its inhibitory effects on implantation in the rat by virtue of its anti-oestrogenic properties and not because of an anti-metabolic action. In the present study we found that fetuses obtained from blastocysts exposed to CI-628 were significantly heavier than those in the other groups while the placental weights remained unaffected. Fetal weight is known to be increased when the litter size is decreased (McKeown, Marshall & Record, 1976) and there were fewer fetuses after exposure to CI-628, perhaps permitting better access of nutrients (Healy, McLaren & Michie, 1960). However, Callantine *et al.* (1966) found no effect on fetal growth and development in rats treated with CI-628 for 13 days. Mouse blastocysts exposed to CI-628 for 24 h exhibited normal development when assessed in terms of their expansion *in vitro* (Sengupta, Dey & Dickmann, 1977), and in the present study exposure to CI-628 and oestradiol-17 β had no effect on subsequent proportions of viable fetuses. The evidence therefore indicates that CI-628 is not embryotoxic and its inhibitory effects on implantation are probably mediated through an anti-oestrogenic mechanism, supporting our view that oestrogen released from the blastocyst is essential for implantation. Oestrogen could enhance a local epithelial oestradiol-17 β receptor translocation to the nucleus (Logeat *et al.*, 1980) and such binding could lead to the release of secondary messenger molecules such as histamine and prostaglandins which have been shown to be involved in implantation in rodents (Kennedy, 1977; Dey, Johnson & Santos, 1979; Johnson & Dey, 1980).

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