Effect of an oestrogen synthesis inhibitor, 1,4,6-androstatriene-3,17-dione, on mouse embryo development *in vitro*

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Summary. Mouse embryos (4–8 cells) were cultured for 48 h *in vitro* in medium containing 1,4,6-androstatriene-3,17-dione (ATD). The inhibition of embryo development by ATD was overcome by oestradiol-17 β but not by oestradiol-17 α . Culture with ATD for the first and second 24 h led to normal numbers of blastocysts. The results show that preimplantation mouse embryos contain the enzymic machinery for synthesis of oestrogen.

Introduction

The development of a fertilized egg to an implanting blastocyst involves a series of structural and biochemical changes during which cellular differentiation into an inner cell mass and trophoblast occurs. Histochemical evidence of Δ^5 -3 β -hydroxysteroid dehydrogenase (3 β -HSD) and 17 β -hydroxysteroid dehydrogenase (17 β -HSD) in mouse preimplantation embryos suggests that embryos may be able to synthesize steroids; maximum amounts are present just before implantation (S. K. Dey & Z. Dickmann, unpublished). The conversion of [3 H]testosterone to [3 H]oestradiol-17 β is also maximal in rabbit blastocysts at this time (George & Wilson, 1978). Steroidogenesis in morulae and blastocysts may play a role in embryonic development and in the preparation of the endometrium for implantation. In the mouse, the development of morulae into blastocysts *in vitro* was inhibited by CI-628 citrate, an oestrogen antagonist (Sengupta, Dey & Dickmann, 1977; Roy, 1980). The present study was undertaken to examine the effect on mouse embryos grown in culture of 1,4,6-androstatriene-3,17-dione, an inhibitor of oestrogen synthetase (aromatase) in human placental (Schwarzel, Kruggel & Brodie, 1973) and rat ovarian (Brodie, Schwarzel & Brodie, 1976) microsomal systems.

Materials and Methods

Random bred Swiss mice were killed on the morning of Day 3 of gestation (Day 1 = day of finding copulatory plug at normal oestrus) to recover embryos. The embryos (80–85% at the 8-cell stage, the rest at the 4-cell stage) were pooled, washed in culture medium and then randomly distributed to tubes for culture for 48 h according to the method described by Whitten (1971). The tubes contained the following substances.

Group 1: 50 μ l ethanol saline (0.9% NaCl solution; 1.4×10^{-2} mmol ethanol) and 4 μ l propylene glycol (5.2×10^{-2} mmol).

Group 2: 4 μ g 1,4,6-androstatriene-3,17-dione (ATD: Research Plus steroids, Denville, New Jersey, U.S.A.; 3.5×10^{-2} mmol) in 4 μ l propylene glycol and 50 μ l ethanol–saline.

Group 3: 4 µg ATD and 1 µg oestradiol-17β (Sigma, St Louis, Missouri, U.S.A.; 3.4×10^{-3} mmol) in 50 µl ethanol-saline.

Group 4: 4 µg ATD and 1 µg oestradiol-17α (Sigma; 3.4×10^{-3} mmol) in 50 µl ethanol-saline.

Group 5: 4 µg ATD for the first 24 h of culture and then no steroid for the second 24 h of culture.

Group 6: 4 µg ATD during the second 24 h of the culture period.

In Groups 5 and 6, embryos were recovered after culture for 24 h, washed in fresh medium and briefly examined under a stereo-zoom microscope to check their development, and then re-cultured in fresh medium.

In each group, cultures were repeated several times (see Table 1) on several days and the development of expanded blastocysts without any apparent signs of abnormality was taken to indicate normal embryogenesis.

In preliminary experiments 1 µg oestradiol-17β/ml was the optimum steroid concentration required to overcome the inhibitory effect of ATD on embryonic development *in vitro*. ATD at a concentration lower than 4 µg/ml was unable to exert any effect on mouse embryo development.

All results were subjected to a statistical test of proportion (Downie & Heath, 1970).

Results

The results for Groups 1 and 3 were similar in that oestradiol-17β overcame the inhibitory influence of ATD (Table 1). However, addition of oestradiol-17α failed to maintain embryonic development. In Groups 2 and 4 the embryos that did not develop were arrested at the morula stage and showed no signs of degeneration. The treatments for Groups 5 and 6 yielded similar high proportions of developing blastocysts (Table 2).

Table 1. Effects of 1,4,6-androstatriene-3,17-dione (ATD) and oestrogens on mouse embryo development *in vitro*

Group	Steroids in culture medium	Steroid dose/ml	No. of 4–8-cell embryos cultured (no. of replicates)	No. of blastocysts developed after 48 h (%)	No. of degenerating embryos
1	—	—	96 (10)	80 (83)	16
2	ATD	4 µg	79 (9)	8 (10)	71
3	ATD + oestradiol-17β	4 µg + 1 µg	96 (9)	73 (76)	23
4	ATD + oestradiol-17α	4 µg + 1 µg	82 (6)	7 (8)	75

Propylene glycol (the solvent of ATD) and 50 µl ethanol (the solvent for oestrogens) were added to the medium in all groups.

Group 1 versus Group 2 and Group 2 versus Group 3: $P < 0.001$; Group 1 versus Group 4: $P < 0.05$ (proportion tests, Z).

Table 2. Effect of ATD given for only 24 h during the 48 h culture period on mouse embryo development

Group	First 24 h			Second 24 h		
	ATD (µg/ml)	No. of embryos cultured	No. of morulae developed	ATD (µg/ml)	No. of morulae cultured	No. of blastocysts developed (%)
5	4	73	73	—	73	58 (73)
6	—	63	63	4	63	53 (84)

Discussion

Culture of mouse embryos with ATD inhibited their development and differentiation. We suggest that ATD interferes with endogenous oestrogen synthesis because the inhibitory effect of ATD on blastocyst development was reversed completely by exogenous administration of oestradiol-17 β . Barker & Anderson (1968) observed that oestradiol-17 α can bind to chromatin of uterine cells, but can not evoke the characteristic responses of hyperaemia and vasodilation normally observed with oestradiol-17 β . In the present study we found that oestradiol-17 α was also unable to mimic the action of oestradiol-17 β on embryonic development.

ATD is an androgenic compound and it could be argued that its deleterious effect on embryos was due to an excessive amount of this steroid. Although ATD has no significant hormonal activity when tested in a conventional bioassay, Christensen & Clemens (1975) noted some androgenic effect in the very sensitive penile spines of the immature rat. In the present study it is unlikely that inhibition is due to any non-specific toxicity or androgenic effect because oestradiol-17 β was effective in nullifying the action of ATD on preimplantation embryos. In rats ATD has an antifertility effect by preventing mating and ovulation and this can be reversed by treatment with exogenous oestrogen (Brodie, Wu, Marsh & Brodie, 1979). Blastocyst formation in Group 5 could have been due to oestrogen biosynthesis by newly formed enzymes or because of the breakdown of ATD into its metabolites which no longer bind to aromatase. In Group 6 the results are explicable if oestrogen is produced in pre-morula stage embryos. Steroidogenic enzymes have been histochemically demonstrated in 4–8-cell embryos (S. K. Dey & Z. Dickmann, unpublished) and oestrogen synthesized in embryos during the pre- or post-morula stages seems to be sufficient for preimplantation embryo development. When this was blocked by exposure of eggs to ATD for 48 h, morula to blastocyst transformation was prevented. These findings suggest that the availability of oestrogen in blastomeres is essential for morula to blastocyst transition.

Rabbit blastocysts are reported to synthesize oestradiol-17 β (George & Wilson, 1978; Gadsby, Heap & Burton, 1980) but no such data are available for the mouse blastocyst. The present study has shown that the mouse preimplantation embryo contains the enzymic machinery for synthesis of oestrogen and that oestrogen is involved in the morula to blastocyst transformation *in vitro*.

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