Effect of human uterine flushings collected at various stages of the menstrual cycle on mouse blastocysts *in vitro*

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Summary. Mouse blastocysts were cultured *in vitro* in a defined medium supplemented with uterine flushings (containing 500 µg protein/ml) obtained from normal women at various stages of the menstrual cycle. With one exception (uterine flushing collected on the last day of a menstrual period) blastocyst hatching and attachment were not impaired by flushings collected before or after ovulation.

Introduction

Implantation in the rat and mouse is dependent upon the close synchronization of embryonic and endometrial development (Noyes, Dickmann, Doyle & Gates, 1963). In these species, as well as in the rabbit (Chang, 1950), sheep (Moore & Shelton, 1964), cow (Rowson, Moor & Lawson, 1969) and pig (Webel, Peters & Anderson, 1970), the uterus exhibits only a short period of receptivity towards the blastocyst. When synchronization is disturbed by more than ± 2 days (Adams, 1976), implantation rarely occurs.

These findings have formed the basis for a current approach to contraception, which aims at the development of pharmacological agents to accelerate the rate of ovum transport in women (Harper & Pauerstein, 1976). However, it has not yet been demonstrated that the human uterus exhibits only a limited period of receptivity towards the blastocyst. One of the many physical and biochemical factors presumed to be relevant to the fate of blastocysts *in utero* is the composition of the uterine luminal fluid. This fluid may contain substances detrimental to blastocysts during part of the oestrous cycle (Chang, 1955) or exhibit embryotrophic properties at the time of implantation (Beier, 1974).

The purpose of the present study was to assess the effect of human uterine flushings on blastocysts *in vitro*. Because there exist at least some similarities between the blastocysts from different eutherian mammals (Brinster, 1973), and because an investigation with human material was not feasible, mouse blastocysts were used.

Materials and Methods

Uterine flushings were collected from normal, parous women at various stages of the menstrual cycle by the technique previously described (Maathuis & Aitken, 1978). The uterus of each subject was flushed with 10 ml of 0.154 M-NaCl. The processing of samples and the indices used to assess the 6 stages of the cycle were as reported earlier (Maathuis & Aitken, 1978). The degree of contamination with plasma protein in each uterine flushing was calculated (Maathuis & Aitken, 1978) and samples in which the concentration of contaminating plasma protein represented less than 10% of the total protein content were selected. The protein content of these samples was adjusted to 500 µg/ml with 0.154 M-NaCl. Of this solution, 0.1 ml was added to 0.9 ml of modified Brinster's medium containing 3 mg bovine serum albumin (BSA)/ml (Bowman & McLaren, 1970), to give a

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Table 1. Growth of mouse blastocysts in a defined medium supplemented with bovine serum albumin (BSA) and human uterine flushings collected at various stages of the menstrual cycle or with BSA alone

Phase of the cycle	Sample	No. of blastocysts	No. of trials	Hatching		Attachment score	
				Overall (%)	Each trial (%)	Overall (%)	Each trial (%)
Early proliferative	1	57	3	5.3	0, 0, 20.0	0	0, 0, 0
	2	59	2	86.4	83.3, 91.3	30.5	29.2, 32.6
Mid-proliferative	3	53	3	60.4	52.9, 63.2, 64.7	29.2	26.4, 28.9, 34.4
	4	76	4	68.4	60.0, 80.8, 50.0, 70.0	19.1	17.5, 17.3, 25.0, 20.0
	5	67	2	85.1	78.6, 89.7	47.0	46.4, 47.4
	6	58	3	91.4	92.0, 83.3, 92.6	45.7	46.0, 41.7, 46.2
Late proliferative	7	81	3	67.9	59.5, 68.2, 81.8	19.4	17.6, 9.1, 33.0
	8	83	3	88.0	95.0, 76.1, 92.3	49.4	51.3, 48.3, 46.2
Mid-secretory	9	25	2	64.0	70.0, 60.0	16.0	10.0, 20.0
	10	69	4	76.8	81.8, 71.4, 76.2, 73.7	37.7	35.2, 42.8, 41.7, 34.2
	11	62	2	91.9	96.7, 87.5	44.4	45.0, 43.7
	12	72	3	91.6	93.3, 89.3, 93.1	42.4	43.3, 39.3, 44.8
	13	76	3	59.2	50.0, 62.9, 68.4	30.3	25.0, 33.3, 34.2
	14	31	2	80.6	83.3, 71.4	37.9	37.5, 39.3
Late secretory	15	61	3	85.2	77.8, 84.2, 100	35.2	33.3, 34.2, 40.0
BSA							

final concentration of 50 µg uterine flushing protein/ml culture medium. Supplementation with BSA was necessary because the neat uterine flushings contained concentrations of protein too low to support blastocysts *in vitro*. This concentration was found in other experiments to be sufficient to permit mouse blastocyst development. The blastocysts were collected 3.5 days *p.c.* from mice of the random bred Q strain and cultured under paraffin oil in 0.05 ml droplets of medium at 37°C in 10% CO₂ in air. Each day the culture dishes were examined microscopically and the number of blastocysts which had hatched from their zona pellucida was determined. An attachment score was obtained by determining the number of blastocysts attached to the substratum (1 point), exhibiting giant cell transformation (1.5 points) and showing extensive outgrowth (2 points). The final result was expressed as a percentage of the maximum possible score (number of blastocysts × 2; Aitken, 1977).

Results

The results are presented in Table 1. One sample (No. 1), collected from a subject on the last day of menstruation, appeared to be embryotoxic because blastocyst hatching and attachment were consistently inhibited. No marked variation in the effect of the remaining uterine flushings on the behaviour of blastocysts could be detected: hatching and attachment scores in uterine flushings collected before (proliferative phase) and after ovulation were not affected ($P = 0.675$ and 0.757 respectively, Wilcoxon's rank test; Snedecor & Cochran, 1967). Culture medium supplemented with bovine serum albumin gave hatching and attachment scores similar to those in media supplemented with uterine flushings and BSA.

Discussion

The results of this study seem to indicate that human uterine fluid does not contain substances embryotoxic for mouse blastocysts during most of the menstrual cycle. The effects observed with Sample 1 may be related to lysosomal enzymes known to be released into the uterine lumen during menstruation (Israel, Mishell & Labudovich, 1970). The reproducibility of the results obtained with Sample 1 suggests that the properties exhibited by the other samples cannot be attributed to the removal or destruction of certain embryotoxic substances during the handling or storage of samples. In addition, similar techniques have been used to demonstrate the embryotoxic nature of uterine fluid recovered from IUD-bearing rats and mice (Breed, 1973).

Mouse blastocysts are known to be fairly undemanding of the conditions for development *in vitro*, but if the properties of the human luminal fluids in this study are indicative of uterine receptivity towards the human blastocyst, then the results suggest that implantation could occur in the human uterus over much of the luteal phase. This may explain why conception seems to be possible after transplantation of human ovaries to the uterine cavity (Estes & Heitmeyer, 1934; Preston, 1953).

Our results do not necessarily lead to the conclusion that contraceptive methods aiming at interference with ovum transport in women may prove to be ineffective. Many other factors, such as myometrial activity (Adams, 1976), the organization of the endometrial luminal epithelium and stromal tissue, and blastocyst-endometrial contact (Psychoyos, 1973), undoubtedly play a part in the process of implantation. Nevertheless, it is clearly necessary to establish the duration of endometrial receptivity towards the blastocyst in non-human primates as a prelude to the search for pharmacological agents that accelerate the rate of ovum transport in women.

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