J. Reprod. Fert. (1978) **53**, 99–101

Effects of progesterone and oestradiol-17β on the spontaneous meiotic maturation of mouse oocytes

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Summary. The spontaneous meiotic maturation of cumulus-free oocytes was not affected by progesterone or oestradiol-17β at 2 μg/ml, but both steroids decreased polar body production at 10 μg/ml and progesterone decreased the number undergoing germinal vesicle breakdown.

Introduction

When mammalian oocytes are removed from antral follicles and are placed into a suitable culture medium, the oocytes undergo a spontaneous meiotic maturation: the arrest of the oocytes at prophase I of meiosis is broken and meiotic division proceeds (Edwards, 1965). It seems, therefore, that the antral follicle exerts an inhibitory action on the nuclear events of oocyte maturation. The mechanism of this inhibitory action has been the subject of several investigations. For example, Tsafriri & Channing (1975) have presented evidence that the follicular granulosa cells produce a meiotic inhibitor, probably a peptide of molecular weight $\simeq$ 2000 (Tsafriri, Pomerantz & Channing, 1976). Others have shown that dibutyryl cyclic AMP prevents spontaneous germinal vesicle breakdown in mouse oocytes (Cho, Stern & Biggers, 1974). More recently, McGaughey (1977) has reported that oestradiol-17β inhibits the meiotic maturation of pig oocytes and that progesterone reverses this inhibition. It was suggested that these steroids may play a role in the regulation of oocyte maturation *in vivo*. We have, therefore, re-examined the effects of oestradiol and progesterone on the spontaneous meiotic maturation of isolated mouse oocytes.

Materials and Methods

The oocytes were isolated from the antral follicles of 20-day-old B6D2F$_1$ mice by piercing the follicles with a 26-gauge needle. The cumulus was removed by vigorous expulsion from a Pasteur pipette. The isolation was carried out in the HEPES-buffered medium previously described (Eppig, 1977) which contained either the steroid to be tested or the ethanol solvent. The steroids were dissolved in absolute ethanol and added to vigorously agitated culture medium. The final concentration of ethanol in all cases, including controls, was 0·1 % while the steroids were used at 2 and 10 μg/ml. Only freshly prepared media were used. After the oocytes were collected in the HEPES-buffered medium they were washed three times by serial transfer and then cultured in Whitten's medium (Whitten, 1971). Both the wash and culture media contained the steroids or ethanol solvent. The oocytes were cultured for 24 h at 37°C in an atmosphere of 5% O_2 + 5% CO_2 + 90% N_2. After the culture period the oocytes were examined for germinal vesicle breakdown and polar body production. The results were assessed statistically by χ^2 analysis.

Results

As shown in Table 1, neither oestradiol nor progesterone at a concentration of 2 μg/ml had any effect on germinal vesicle breakdown or polar body production. A concentration of 10 μg oestradiol/ml did not affect the total number of matured oocytes although polar body production was reduced by about 20% ($P < 0.01$). Progesterone at 10 μg/ml decreased the total number of oocytes matured by about 34% ($P < 0.01$) and almost eliminated polar body production. Oestradiol and progesterone together gave the same effect as progesterone alone.

Table 1. Effects of oestradiol and progesterone (2 or 10 μg/ml) on spontaneous meiotic maturation of denuded mouse oocytes

	Controls				Oestradiol				Progesterone				Oestradiol + progesterone			
Exp.	Total	GV	GVB	PB	Total	GV	GVB	PB	Total	GV	GVB	PB	Total	GV	GVB	BP
2 μg/ml																
1	222	15 (7)	207 (93)	88 (40)	169	10 (6)	159 (94)	73 (43)	203	6 (3)	197 (97)	60 (30)	189	11 (5)	178 (95)	32 (17)
2	155	10 (6)	145 (94)	98 (63)	253	8 (3)	245 (97)	161 (64)	229	17 (7)	212 (93)	150 (66)	216	9 (4)	207 (96)	129 (60)
3	242	21 (9)	221 (91)	148 (61)	221	13 (6)	208 (90)	129 (58)	247	20 (8)	227 (92)	147 (60)	170	19 (11)	151 (89)	100 (59)
Total	619	46 (7)	573 (93)	334 (54)	643	31 (5)	612 (95)	363 (56)	679	43 (6)	636 (94)	347 (51)	575	39 (7)	536 (93)	261 (45)
10 μg/ml																
1	234	12 (5)	222 (95)	93 (40)	217	13 (6)	204 (94)	56 (26)	201	42 (21)	159 (79)	7 (3)	221	59 (26)	162 (74)	2 (1)
2	220	18 (8)	202 (92)	68 (31)	224	11 (5)	213 (95)	70 (31)	242	71 (29)	171 (71)	9 (4)	237	80 (34)	157 (66)	10 (4)
3	275	14 (5)	261 (95)	184 (67)	312	14 (4)	298 (96)	85 (27)	297	180 (61)	117 (39)	8 (3)	260	181 (70)	79 (30)	9 (3)
Total	729	44 (6)	685 (94)	345 (47)	753	38 (5)	715 (95)	211 (28)*	740	293 (40)*	447 (60)*	24 (3)*	718	320 (45)*	398 (55)*	21 (3)*

Figures in parentheses are the percentages related to the total number of oocytes used in that experiment. GV = germinal vesicle present; GVB = germinal vesicle broken down; PB = oocytes matured with polar body.

* Indicates a significant difference of that total group from the total control group.

Discussion

These results are the reverse of those obtained by McGaughey (1977) who found that oestradiol blocked the meiotic maturation of pig oocytes and that progesterone reversed this inhibition. In either study, however, it would be presumptuous to suggest that the results are indicative of mechanisms of meiotic inhibition *in vivo*. The concentrations of steroids (2 and 10 μg/ml) used in the present study are within the range found to be effective by McGaughey (1977) for pig oocytes. Concentrations ranging from about 1 to 4 μg/ml of the steroids have been reported to be present in the follicular fluid of preovulatory monkey and human follicles, although being very much lower in that of earlier antral follicles (Sanyal, Berger, Thompson, Taymor & Horne, 1974; McNatty, Hunter, McNeilly & Sawers, 1975; Channing & Coudert, 1976). It is therefore likely that the inhibitory effects of oestradiol or progesterone on spontaneous meiotic maturation are non-physiological. The meaningfulness of experimentally addressing the mechanisms of meiotic inhibition by using spontaneously maturing oocytes may be questioned. Biggers & Powers (1977) have argued that spontaneous oocyte meiotic maturation may be an artefact of culture. At best, spontaneous maturation is only an incomplete maturation since these eggs do not have normal developmental competency (Niwa & Chang, 1975; Thibault, Gerard & Menezo, 1975; Moor & Trounson, 1977), or qualitatively normal protein synthetic patterns (Warnes, Moor & Johnson, 1977).

This research was supported by a grant from the National Science Foundation (PCM7603047). S.L.K. was a participant in the Student Science Training Program of the National Science Foundation (SM177-00641). The Jackson Laboratory is fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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Received 7 October 1977