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Effects of Varying Concentrations of Oestradiol on Embryonic Development

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Summary

Effect of various concentrations of oestradiol was studied on the rat embryos in vitro. In vivo fertilized day-2 embryos from Wister rats were cultured in T6 medium containing 0, 300, 900 and 1500 pg/ml oestradiol (E_2) for 96 h. Embryos were evaluated for their rate of cleavage, fragmentation, granularity, and degeneration at 24h intervals. In the presence of high levels of E_2, in vitro, the ability of 2-cell embryos to undergo further cleavage was compromised in a dose dependent manner and about 6-36% of the embryos did not undergo further cleavage from 2 cell stage while about 53-73% cleaved once and not further. Similarly, embryo quality was affected in 32% of embryos exposed to 300pg/ml E_2 as compared to <5% in the control group.

Introduction

Multiple folliculogenesis following hyperstimulation in assisted reproduction programs normally results in high serum oestradiol levels. The diverse biological and toxic effect of oestrogens may alter the growth pattern during early embryonic development in vivo (Beyer and Juchau 1988, 1989). Therefore, in this experiment, we studied the effect of various concentrations of oestradiol (E_2) on growth and development of pre-implantation rat embryos in vitro.

Materials and Methods

Sexually mature female Wistar rats (200-250g) were used throughout this study. The animals were maintained under natural conditions of temperature (28-30°C), and light (12:12h of light and dark). The animals had free access to food and water *ad libitum*. Mating in pairs was carried out in polypropylene cages. Superovulation was not used to avoid difficulties in interpreting results from its possible effects on embryos. Normal females were caged with fertile male. Successful mating was documented the following morning by the presence of sperms in the vaginal flushing. Day-2 pregnant rats were sacrificed between 1200-1400h using carbon dioxide gas. Oviducts were removed and transferred to the modified T6 medium. The embryos were washed twice in the medium and only morphologically normal 2-cell embryos were then transferred into T6 medium droplets containing 0, 300, 900 and 1500 pg/ml of oestradiol and cultured under mineral oil. The embryos were incubated at 37°C in 5% CO_2 in air at humidified atmosphere for 96h (until day-6 of development). Oestradiol was available in the medium throughout the experiment. The embryos were evaluated under microscope for their rate of cleavage, fragmentation, granularity, and degeneration at 24h intervals.

Results

Results from this series of experiments showed that oestradiol acted directly on the 2-cell rat embryos

thereby affecting their subsequent embryonic development over the next 3-4 days. The direct embryo-toxicity of oestradiol was manifested in two aspects of the development, namely: 1) the rate of growth; and 2) the quality of the embryos.

I. Effect on rate of development:

The growth rate of embryos was affected by exposure in vitro to different concentrations of E_2. On day-3 (i.e. 24h after initiation of culture), about 45% of embryos had progressed beyond 2 cell-stage in the control group. However, in the 300 and 900pg dose groups, only 22% had reached 3-4 cell stage while, in the 1500 dose group only 15% embryos had progressed to the 3-4 cell stage. These values (Table I) were significantly lower than corresponding rate in the control group.

Similarly, on day-4, majority of the untreated embryos (about 65%) had reached the 5-8 cell stage and/or compaction and this was approximately 3-10 fold higher than the treated group. The cleavage rate declined with increasing E_2 concentration and this decline was dose-dependent. At higher doses, the ability to cleave further was found to be irreversibly damaged as evident by the cell stage on Day 5 and 6, especially in the 1500 pg dose group when none of the embryos reach the morula stage (Table I).

II. Effect of β-oestradiol on the embryo quality

Embryo quality was affected by exposure in vitro to different concentrations of E_2. A significant ($p<0.005$) decline in the quality was observed in embryos exposed to 300 pg/ml E_2, 32% as compared to less than 5% in the control group. The majority of the abnormal embryos were degenerated during the course of their in vitro development in culture. Similarly, fragmentation rate was also higher in this group as compared to control (Table II). In the 900pg/ml dose group, abnormal embryos accounted for almost 15%, however, it was not significant when compared to the corresponding rates in the control group. However in the 1500 pg group, the proportion of abnormal embryos was significantly higher than in the control group with degenerated embryos accounting for most of the abnormality (Table II).

Discussion

Most embryos exposed to different concentrations of E_2 failed to develop beyond 3-4 cell stage, especially at higher doses (900 and 1500pg) where the retardation of embryonic development was maximum. A significant number of embryos showed fragmentation and/or degeneration at 300pg. These abnormalities were noted, however, in the later stages of development. However, at 900pg, no significant morphological abnormalities were found. This may suggest that morphological changes may be expressed as a late effect which can be seen only if embryos undergo further division. In contrast to this finding, at 1500pg, a significant number of embryos was degenerated when compared to the control and 900pg group. This could suggest the highly lethal effect of E_2 on the embryos.

The result of the present study confirmed that direct exposure of 2-cell rat embryos in culture was embryo-toxic indicating that high levels of oestradiol acted directly on the embryos to effect its toxic action.

The observations that E_2 treatment induced embryo toxicity at the lowest concentration was in good agreement with the findings of Beyer and Juchau (1987), who reported that oestradiol-17B and 17α-ethinyl oestradiol-17B (EE) altered the growth and developmental patterns of cultured whole embryos during the early stages of organogenesis in rats. It confirmed what was shown in vivo when rats stimulated with PMSG had very low viable pregnancy rate. The exact mechanism of direct E_2-induced embryo-toxicity is not known. It may be produced by conversion of endogenous E_2 to reactive intermediates by P 450 dependent enzyme systems. Akira et al (1993) observed an acceleration in speed of embryo transport and implantation failure in rats superovulated with PMSG, which resulted in increased E_2 level during day 2 and day 3 of pregnancy. Similarly, Cline et al (1977), Beyer and Juchau (1988, 1989) reported the adverse effect of oestrogens on various mammalian systems. Our in vitro results confirmed the suggestions of these in vivo studies. The major action of E_2 is mediated by the oestrogen receptors. If the embryonic ERs are physiologically active cellular response to oestradiol will be expressed in the embryos. Receptors for E_2 are detected from the unfertilized oocyte to the early embryos at the 3-4 cell stage in mouse (Wu et al, 1992). The number of ERs declined at the later stages (after 3-4 cell stage) but reappear after compaction (Hou and Gorski, 1993). Hence, in the present study we have used 2-cell embryos which have ERs if rat embryos are similar to mice's. The fact that we have shown that the exposure in vitro produced a dose-dependent effect indicates that ERs most probably present on 2-cell embryos. Most embryos treated with E_2 did not undergo further cleavage beyond the 2-cell stage.

In vivo embryos may normally be protected from oestrogen exposure by variety of mechanisms that limit the concentration of 17β-oestradiol, the oestrogen that is

Table I: Development of rat embryos exposed to varying concentrations of oestradiol in vitro from 24 to 96h (until day-6 of development) in culture

	Group	E_2 (pg/ml)	Number (%) of embryos at different development stages				
			2 cell	3-4 cell	5-8 cell	Morula	Blastocyst
Day-3	1	0	51 (54.2)	40 (42.6)	3 (3.2)	0	0
	2	300	52 (77.6)	15 (22.4)	0	0	0
	3	900	37 (77.1)	11 (22.9)	0	0	0
	4	1500	45 (84.9)	8 (15.1)	0	0	0
Day-4	1	0	2 (2.1)	29 (30.9)	46 (48.9)	17 (18.1)	0
	2	300	7 (10.4)	46 (68.7)	14 (20.9)	0	0
	3	900	14 (29.2)	33 (68.7)	1 (2.1)	0	0
	4	1500	18 (34.0)	30 (56.6)	5 (9.4)	0	0
Day-5	1	0	1 (1.1)	11 (11.7)	17 (18.1)	44 (46.8)	21 (22.3)
	2	300	5 (7.5)	45 (67.2)	6 (8.9)	11 (16.4)	0
	3	900	8 (16.7)	37 (77.1)	3 (6.2)	0	0
	4	1500	17 (32.1)	31 (58.5)	5 (9.4)	0	0
Day-6	1	0	1 (1.1)	14 (14.9)	11 (11.7)	10 (10.6)	58 (61.7)
	2	300	4 (6.0)	41 (61.2)	8 (11.9)	4 (6.0)	10 (14.9)
	3	900	8 (17.0)	35 (74.5)	4 (8.5)	0	1
	4	1500	19 (35.9)	28 (52.8)	6 (11.3)	0	0

Fisher's exact test:- group-1 is significantly different from all other groups in 3-4 cell stage on day-3, 5-8 cell stage on day-4, morula on day-5 and blastocyst stage on day-6.

Table II: Morphological abnormalities developed in the embryos after exposure to various concentrations of oestradiol

E_2 (pg/ml)	No. embryos cultured	Normal (%)	Abnormal embryos			
			Fragmented	Granulated	Degenerated	Total (%)
0	94	90 (95.7)	2	1	1	4 (4.3)
300	67	45 (67.2)	7	2	13	22* (32.8)
900	48	42 (87.5)	1	1	4	6 (12.5)
1500	53	38 (71.7)	1	0	14	15* (28.3)

Fisher's exact test: values of total abnormal embryos are significantly sifferent from each other ($p<0.05$) except between groups*.

normally found in most species around the developing embryos (Gorski and Hou, 1995). However, embryonic development can also be disrupted by reactive metabolic intermediates of variety of chemicals, suggesting that oestrogens could further influence embryogenesis via receptor-independent mechanism (Juchau, 1989).

Oestrogens is needed for implantation in mouse and other species (Yoshinaga and Adams 1966). However, its excessive production during early embryonic development - due to ovarian hyperstimulation may be detrimental to the embryos. The toxic effect on the embryos observed in our in vitro study supports the direct toxic effect of oestrogens on early embryos during the preimplantation period.

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