Transplacental Administration of Diethylstilbestrol in Rat: Effect on Descendants' Fertility

HATLARA TRANSPLASENTAL DİETİLSTİLBESTROL VERİLMESİNİN YENİ JENERASYONUNUN FERTİLİTESİNE ETKİSİ

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SUMMARY

In order to determine the effects of transplacental exposure to diethylstilbestrol (DES) on the descendants' fertility, pregnant Sprague Dawley OFA rats were treated with DES (10, 20, 50 and 100 yg/kg/d, subcutaneous) from day 10 to 18 of gestation (long time) or day 13 to 18 of gestation (short time). Only the group given the lowest doses (10 \ig/kg/d, short and long times, and 20 \ig/kg/d, short time) gave birth to alive descendants. The fertility of the male and female descendants was determined postnatally by a breeding technique and appeared to be strongly decreased among females of all groups (70 to 100 % of sterility) and moderately among males (11 to 18 % of pathological fecundity). These rats with pathological fecundity gave birth to descendants whose fertility appeared to be normal.

KeyWords: OES-rat-infcrlility

Anatolian J Gynecol Ohst 1992,2:51-56

ÖZET

Bu çalışmada, DES'un üreme sistemi üzerine olan etkisi araştırılmıştır. Bu amaçla, gebe Sprague Dawley; OFA farelere, DES supkutan olarak günde 10, 20, 50 ve 100 mikrogram/kg, olmak üzere, gebeliğin 10-18 günleri arasında (long time) ve gebeliğin 13-18 günler arasında (short time) aynı dozlarda uygulanmıştır. Sadece düşük doz verilen grup (10 lig/kg/günde; kısa yada uzun süre ile 20 ug*/kg/günde kısa süre alanlar) canlı sağlam yavrular doğurmuşlardır. Erkek ve dişi yavruların fertiliteleri postnatal üreme teknikleri ile tespit edilmiş ve dişilerde % 70-80 nisbetinde sterilite, erkeklerde ise % 11-18 oranında patolojik fekondite görülmüştür. Bu patolojik fekonditeli far eler de fertilitesi normal görülen fareler doğurmuştur.*

Anahtar Kelimeler: DES-rat-infertilitc

T Klin Jinekol Ohst 1992,2:51-56

Diethylstilbestrol (DES), a synthetized oestrogen (DODDS and al , 1938), has been very largely used since 1945 in order to prevent some pregnancy complications (gravidic toxemia, in utero mortality, premature delivery). The number of women treated in this way during their pregnancy between 1945 and 1971 is estimated at two to three millions in the United States and at approximately three hundred thousand in France.

Inefficiency of DES in the above-mentioned indications was demonstrated in 1953 by DIECKMAN's work and in 1970 HERBST and SCULLY brought to the fore the risks of vaginal adenocarcinomas in the girls whose mothers had been treated by DES during their pregnancy. This discovery led the Food and Drug Admiristration to forbid this product in 1971, but its use for other indications was

Geliş Tarihi: 08 11.1991 **Kabul Tarihi: 13.11.1991**

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again authorized in 1975. In France its use completely disappeared only in 1977. These treatments' repercussions in the children exposed to DES in utero are at present frequent in human gynaecology and one can consider it will only be around the year 2000 that most of the troubles will have been observed in these descendants.

Though vaginal adenocarcinomas' risks are very rare, Ok, 14 to 1,4 cases/1000 exposed subjects (HERBST, COLE and COLTON, 1977), other hannful effects on reproduction may be observed.

BLACKBILL and BERENDES, in 1978, demonstrated that the number of miscarriages was multiplied by two and premature birthgivings by three in the exposed women. A lot of other publications (BIBBO and al., 1977; COUSINS and al., 1980; STILLMAN and al., 1982; BELAISCH, 1983; DRAPIER, 1984) specified these hannful effects, proving particularly the role of DES in the appearance of malfonuations of the uterus, of the Fallopian tube and of the cervix and vagina (SANDBERG, 1976; KAUFMAN and al., 1977, 1980). These malfonuations consume a very important negative factor for exposed women's pregnancies, since only half of these women carry their pregnancy through to completion against 85 % for the control women.

Apart from these reproduction troubles connected with morphological abnormalities of the genital system, it seems that there are some cases of primary infertility linked to DES administration which are observable in men as well as in women that are exposed in utero.

The aim of this experimentation was to obtain an animal model (rat) that is subfertile after in utero exposure to DES and to study:

—the importance of subfertility

—the repercussions of this exposition as regards physiology, anatomy and histology.

MATERIALS AND METHODS

l.ANIMAL MAINTENANCE AND TREATMENT

1.1. Treatment of the parental generation (I)

Virgin Sprague-Dawley female rats (200-250 g; OFA) were maintained at 22°C under constant 12 L:12 D cycle with free access to food and tap water. Pregnancies were generated by placing a female with a fertile male in the late afternoon. The day a vaginal plug was discovered was designated day 0 of pregnancy. Forty-eight females were treated and eight groups of six other females were housed as control animals. Pregnant experimental rats were randomly divided into 4 treatment groups: 10 pg;20 ug; 100 ug/kg/day (Table 1). They were daily treated with a subcutaneous injection of DES in olive oil either from day 13 to 18 or from day 10 to 18 of gestation. Control females were given injections of olive oil on the same days. The weight and general health of each pregnant animal were recorded daily. After delivery, pups were placed with an untreated foster mother and were daily weighed.

1.2. Treatment of the exposed females (generation II)

Female descendants of the parental generation were housed, mated like the first generation and gave birth to a third generation.

2. FEC UNDITY OF THE DESCENDANTS

2.1. Fecundity of the females (generation II)

The study concerned different criteria:

—puberty appearance: detennined by vaginal opening (physologically: 37th day+ 2);

—the regularity of the cycles and oestrus' progress (daily vaginal smears);

—fertility evaluation: it was estimated by the number of newborns obtained per female.

2.2. Fecundity of the males

Table **1.** Treatment period and doses of des

DES Ug/kg/day	TREATMENT PERIOD short time (ir/-18ᶜ jour)	long time (13'-18ᶜ jour)
100Ug/kg/d	s too	L 100
50Ug/kg/d	S 50	L50
20iig/kg/d	S 20	L20
10Ug/kg/d	S 10	L 10

Exposed males were mated with control females and their fecundity as well as that of the females was studied.

3.STATISTICAL ANALYSIS

The threshold of subfertility was determined from the control litters by a statistical method (REED and al., 1971). Threshold: below 10 newborns/litter.

4. ANATOMY AND HISTOLOGY OF THE GENITAL TRACTS

Evidence of teratogenic effect of exposure to DES was looked for in male and female descendants. An anatomical and histological study (optic and scanning microscpy) was carried out on the genital systems.

RESULTS

1. FECUNDITY OF THE TREA TED FEMALES (Generation I)

In all the groups, miscarriages, cannibalism or a very important rate of stillbirths were noted. At the highest doses (50 pg/kg and 100 ug/kg) no litter survived more than a day. Only the groups S20, S10, L10 had descendants (Table 2). These products were apparently normal despite a birth weight lower than that of the control animals.

2. FECUNDITYOF THE EXPOSED FEMALES (Generation IT)

—Puberty's appearance was delayed in the groups exposed to 10 to 20 pg/kg (Table 3).

—The sexual cycles were not altered except for the 10 females from the group 10 pg/kg/day in which an oestrus' lengthening of 12 to 48 hours was noticed.

Table 2. Fecundity of the treated females (generation I)

Groups	Numlter of litters	Numtx-r of newborns	Fertility*
S 20	**6**	**48**	**8**
S 10	**8**	**88**	**11**
L 10	**6**	**59**	**9,8**
Controls	**19**	**283**	**14,8**

***Fertility:number of newborns/females**

Table **3.** Age of puberty in the exposed females (generation II)

Groups	Number of litters	Number of females	Puberty (in days) average	Sin*	**Student's** test/Controls
S 20	**6**	**29**	**40**	1,76	**1-4**,48 p<0,001
S10	**8**	41	40,!	1,22	NS
L 10	**6**	31	39,5	**0**,99	**NS**
Controls	19	127	**39**	0,41	

***dcviation/avcrage**

Table4. Fecundity Of the in utero exposed females (first pregnacy)

	S 20	S 10	L 10	Controls
Number of Females				
normal fertiliy	**0**	6	5	61
subfertly	9	**8**	**8**	**2**
sterility	**11**	**10**	6	**0**
		24	19	**63**
Percent of animals				
normal fecundity	0	25	26	97
***Pathological fecundity**	too	75	74	3

***patliologieal fecundity (subfertilitysterility)**

2.1. Fecundity during the first gestation

The repercussions on fecundity during the first gestation are represented on Table 4. An impairing of fecundity was established in 70 to 100 % of the cases.

2.2. Evolution of the exposed females' fecundity

The exposed females underwent a cycle of 5 successive gestations separated by resting periods of 8 days. The fecundity results (Table 5) show that the subfertility or sterility of the females is definitive.

2.3. Fecundity of the descendants of the exposed females (Generation III)

The hypofertile females gave birth to a third generation whose fecundity appears entirely normal (Table 6).

TableS. Fecundity of the in utero exposed females in the following pregnancies

Groups	jst pregnancy	pregnancy	3 ^ pregnancy	4 ^ pregnancy
S 20				
N	**0**	**0**	**0**	**0**
P	too	**100**	**100**	**100**
S 10				
N	**20**	**28**	**25**	**30**
P	**80**	**72**	**75**	**70**
L 10				
N	**22**	**0**	**0**	**0**
P	78	**100**	**100**	**100**
Controls				
N	**97**	**97**	**%**	**97**
P	**3**	**3**	**4**	**3**

N-normal fecundity
P • pathological fecundlily (subfertility and sterility)

Tableé. Fecundity of the exposed females' descendants (generation **III)**

	G mops	number of litters	number of newborns	fertility*
male descendants	**S 10**	**12**	**164**	**13,7**
	L 10	**12**	**143**	**11,9**
female descendants	**S 10**	**10**	**130**	**13,0**
	I, 10	**12**	**160**	**13,3**

***fertility:nıımlx-r of newborns/femalcs**

3. FECUNDITY OF THE EXPOSED MALES (Generation II)

Table 7 shows that whatever the dose and the duration of the treatment may be, the impairing of fecundity is less important than in females (18 % of pathological fecundity).

4, ANATOMICAL AND HISTOLOGICAL REPERCUSSIONS OF EXPOSITION TO DES

4.1. In males

No case of true cryptorchidism was noticed but a lot of males presented a great testicular mobility.

Testicular hypotrophies were mainly put to the fore in the exposed males (gruop Sio: 11 %; group Lio: 31 %). They were always accompanied by a compensatory hypertrophy on the opposite testicle.

The examination of the different segments of the male genital apparatus in optical microscopy allowed to show histological modifications: deterioration of the spermatic stock, histological impairing of the seminal vesicles.

4.2. In females

Some very rare uterine malformations (1/20) were observed as well as one single cervical lesion which appeared to be of an inflammatory nature and could be due to the numerous vaginal smears that were achieved. Neither ovarian malformations nor polymicrocystic ovaries nor abnormal follicles were observed.

DISCUSSION

This work shows that in utero exposure of Sprague Dawley rats to DES doses ranging from 10 to 20 pg/kg/day 5 or 8 days leads to a very significant diminution of fertility in male and in female compared with the untreated animals.

The effects observed on the subcutaneously treated females (miscarriages, stillbirths, cannibalism) had not been mentioned in the fonuer works (RUSTIA and SHUBIK, 1976; WALKER, 1980, NOMURA and MASUDA, 1980, McLACHLAN, 1982; WALKER, 1983, BOYLAN and al., 1983). This can be explained by differences:

—either between species: mice are utilized by most of the authors except for RUSTIA and SHUBIK (1976) and HENDRY and LEAVITT (1982)

Table 7. Fecundity of the exposed males

	S20	S10	L 10	Controls
Number of Males				
normal fertility	**15**	**40**	**22**	**86**
subfertility	**3**	**4**	**2**	**4**
sterility	**0**	**1**	**3**	**0**
	18	**~Ï5~**	**27**	**~9Ö~**
Percent of animals				
normal fecundity	**83**	**89**	**82**	**96**
'pathological fecundity	**17**	**11**	**18**	**4**

***pathological fecundity (subfertility and sterilily)**

who use hamsters as well as BOYLAN and al. (1983) who use rats;

—or between doses (from 0,01 Jig/kg to 1 mg/kg, or even 40 mg/kg, orally, in RUSTIA and SHUBIK (1976));

—or between administering ways and between galenic forms: DES was administered either orally (RANDS and al., 1982), or subcutaneously in an oily solution (McLACHLAN and al., 1982; WALKER, 1983; NEWBOLD and al., 1983), or DES salts subcutaneously in an aqueous solution (NOMURA and MASUDA, 1980);

—or between duration of administration (from a single administration to daily administration during a certain period of gestation). However McLACHLAN and al.'s work (1982) in mice, the closest to ours withregard to dosage, duration and way of administration, mention only 50 % of miscarriages or resoptions with a dose of 100 mg/kg whereas we did not obtain any birth with dose nor with a dose of 50 ug/kg. The important cannibalism that we observed may be explained by a persistence of the estrogenic impregnation which would inhibit prolactin secretion and therefore lactation beginning.

—The effects observed on the descendants are also different from the previous studies:

Contrarily to the observations of BOYLAN (1978), McLACHLAN and al. (1982), there is no puberty appearance precocity in female rats exposed in utero to DES 10 ug for a long-lasting period, but on the contrary a significant delay at the dose of 20 ug/kg. The only modification noticed in the cycle's development was an extension of oestrus' duration (48 hours instead of 12 hours) at a dose of 10 pg/kg (the cytological interpretation may have been hindered by an important keratinisation of the vaginal epithelium which was already observed by McLACHLAN and al. (1982) in mice.

As for the in utero exposed females (generation II), all groups presented subfertility or sterility at a percentage varying, according to doses and treatment's duration, from 70 to 100 %. Amongst the possible explanations for this subfertility, neither modifications of sexual behaviour nor ovulation's disorders seem to be agreeable ones because ai! the females were **normally** cycled and accepted , e ring.

The effect of in utero exposure is less definitive in males. At a dosage of 10 pg/kg administered on a long time treatment, 18 % of the males are subfertile. Fertility with a 20 pg/kg administration in a longlasting treatment should be checked for the group 20 pg/kg unfortunately did not give any descendants.

This fecundity impairing does not seem to have an anatomical or structural origin because the malformations of the genital system that have been noticed are very inconstant and very moderate as it was recently confirmed by ROTSCHILD and al' s work (1988). The functional consequences seem to be major in comparison with the structural and morphological changes which aie far more modest than the ones observed in man for a similar exposition; so another etiology has been looked for by detennining the number of the endometrial receptors to oestradiol and progesterone (COGNAT and al., 1990). The impairing of fecundity by transplacental exposure to DES does not pass on to the following generation as it was shown in this work.

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