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Reduction of Estradiol Receptor in Rat Atretic Follicle Induced by Dihydrotestosterone Enanthate (DHTE)

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DHTE로 유발된 흰쥐 폐쇄난포의 에스트라디올 수용체의 감소

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요 약

Dihydrotestosterone (DHT)은 난소내 폐쇄를 유발한다고 보고 되었으나, 반대로 폐쇄를 억제한다는 상반된 주장이 있어 왔다. 본 연구는 난소내 난포가 폐쇄되는 기작을 밝히는 연구의 일환으로 흰쥐에 DHT를 처리하여 난포가 폐쇄되는지 여부와 폐쇄된다면 스테로이드 수용체, 특히 에스트라디올 수용체에 어떠한 영향을 주는지를 조사하고자 하였다.

미성숙 흰쥐의 뇌하수체를 절제(HPX)하고, 35IU의 PMSG 또는 PMSG 처리후 Dihydrotestosterone enathate (DHTE)를 lmg-5mg/kg 체중 농도로 처리하여 난포의 폐쇄여부를 조직학적으로 검경하였다. PMSG, PMSG-DHTE 공히 강소를 형성한 난포의 acid phosphatase 활성도를 증가시켰고, 또한 난포의 폐쇄를 유발하였다. 흰쥐의 난소내 estrogen receptor (ER)의 수는 과립세포에 가장 많이 분포되어 있었고, HPX로 가용한 수용체의 수가 증가하였으며, 과립세포에서도 세포질내 ER은 핵내로 이용되는 것을 확인하였다. 또한 DHTE는 난소내 ER의 농도를 감소시켰다. 이 결과로 보아 난포내 DHTE는 ER의 농도를 감소시켜 폐쇄를 유발하는 요인으로 작용할 것이라는 결론을 얻었다.

INTRODUCTION

Follicular atresia is one of the universal and characteristic phenomena in the mammalian ovary. Genarally it is estimated that greater than 99 percentage of the ovarian follicle become atretic but the initiation mechanism of follicular atresia is not clear yet.

Estrogen plays a major role in follicular growth and development, proliferation of granulosa cell (GC), and production of progesterone(P). Estrogen also synergizes with gonadotropins to cause the maturation of follicle in preparation for ovulation (Richards, 1980, 1988). In the mammalian ovary, mainly of human or rat, estrogen receptors (ER) have been found in granulosa cells (GC) as well as in theca cells (TC), and luteal cells (Richards *et al.*, 1980, 1987). On the other hand, androgens antagonize the growth promoting action of estrogen in the ovarian tissue and bring about the reduction in ovarian weight (Payne and Runser, 1958; Louvet *et al.*, 1975; Saiduddin and Zassenhaus, 1978; Azzolin and Saiduddin, 1983), but its receptor is not changed during atresia (Yoon *et al.*, 1989b). These studies suggested that androgens may not act through its receptor in the follicle maturation and atresia.

The higher level of androgen and the lower

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level of etrogen in ovarian follicular fluid (FF) are associated with the atresia of the mammalian follicles (McNatty *et al.*, 1979; Bomsel-Helmreich *et al.*, 1979; Tsonis *et al.*, 1984; Lee and Yoon 1985; Lobo *et al.*, 1985; Yoon *et al.*, 1990). Therefore, it can be assumed that steriod hormones are the intragonadal local regulatory factors for the follicular cell functions during follicle maturation and atretic processes and also for the cumulus enclosed oocytes. However, it is not yet known whether estradiol in follicular fluid (FF) act on the differentiation of ovarian cells through their receptors as an autocrine system or not.

We report here the changes of estradiol(E_2) receptors in the rat atretic ovary induced by prenant mare's serum gonadotropin (PMSG) and dihydrotestosterone enanthate (DHTE) and the notion that androgen dependent decrease of E_2 receptor level might be one of the mechanism in promoting follicular atresia.

MATERIALS AND METHODS

CHEMICALS

The following reagents were purchased from Sigma Chem. Co.: Unlabelled steroids and diethylstillbesterol (DES), Trizma-HCl, dithiothreitol (DTT), EDTA, phenylmethylsulfonyl-fluoride (PMSF), diisopropylfluorophosphate (DFP), sucrose (grade I), DNA from calf thymus, charcoal (Norit A), flutamide(a,a,a-trifluoro-2-methyl-4'-nitro-m-propionotoluidide). Testosterone- and dihydrotestosterone enanthates were kindly donated from Dr. H. K. Kim of NIHCHD. Tritiated moxesterol (R2858, spec. act. 70-87 Ci / mol), $^{2,4,6,7,16,17\text{-}3}$H-estradiol-17 ($^3HE_2$, 130-170 Ci / mmol), and cold R2858 were purchased from New England Nuclear, GmbH. The tritium labelled tracers were assayed for their purities by ascending thin layer chromatography (Merck, 0.25 mm thickness, silica gel) after the addition of unlabelled ligands. The purifying solvent system was benzen / ethyl acetate (3:1, v / v). The spots were identified by brief staining over iodine vopors, scraped off and counted. All other chemicals used were analytical grade unless otherwise stated.

ANIMALS

Immature Sprague-Dawley female rats (21 day old) were supplied from a local agent and exposed to 25℃ under 12D: 12L / cycle giving food and water *ad libitum*. Hypophysectomized (HPX) rats at 24 days of age were maintained on above conditions suppling 10% dextrose in saline solutions *ad libitum*. At age of 30 days, animals were weighed and randomly assigned to one of the three treat regimens: 1) saline and sesame oil after 30 hrs. 2) PMSG (35 IU) and sesame oil, 3) PMSG and DHTE 1 mg / kg of body weight. The first injection of PMSG or vehicle was given at 10:00 AM subcutaneously on day 30 and the second injection of DHTE or vehicle, 30 hrs later at 4:00 PM on day 31. All animals were weighed 24 hrs later, decapitated, and blood was collected. Ovaries and uteri were weighed separately and then quick-frozen for storage in liquid nitrogen or fixed in Bouin solution for routine histologic analysis. Alternative frozen sections (10nm) were treated for enzyme histochemistry for acid phosphatase by a modified method of Pearse (1968) but with an acetate buffer, pH 5.0.

All slides of frozen sections were randomly assigned a code number and five sections were selected for quantitation of lysosomal acid phosphatase (AcP) from each animal in all three treatment groups. A grade 2- positive reaction was assigned if a homogeneous distribution of a bright red color was present in the granulosa cell layer (GCL) of the follicle. A negative reaction (0) was considered to be a dull brown-yellow color equivalent to the color of the stromal tissue on the control slide. To establish follicle development, and numbers, and atresia, every tenth routine histologic section of each ovary was analyzed by

counting the number of follicles exhibiting an oocyte nucleus in cross section. The follicular stage and atresia were assessed by Byskov's morphological criteria for atresia (Byskov, 1978, 1979). Of the developmental classification, the primordial follicles (PF) constituted classes 1 to 3B with up to 60 GC: the preantral follicles (PAF), classes 4 to 5B with up to 400 GC: the antral group, classes 6 to 8 with > 400 GC.

TISSUE PREPARATION

Rats were guillotined and then uteri, ovaries and skeletal muscle rapidly dissected out, where indicated. Rat ovaries were quickly denuded with fine scissors of all extra-ovarian tissues, washed and placed in chilled homogenation buffer as follows. All subsequent procedures were carried out below 0-4℃. The ovaries were placed in embryo dissecting dishes containing the buffer (TEGT), 20 mmol / L Tris-HCl, pH 7.5, 0.3 mM EDTA, 1 mM DTT, 10% glycerol, 12 mM thioglycerol and freshly prepared 1.0 mM PMSF. The follicles randomly punctured with dental 30-gauge needle and the granulosa cells were squeezed out with a pair of fine forceps. Gentle pressure was applied to follicles and the expressed cells were collected after centrifugation at 800 xg (20 min, 4℃). The cells and pelleted GCs from 2 ovaries / tube (0.1 ml volume, for the E_2 nuclear receptor assay) were washed thrice in TEGT and also the remaining ovarian tissues (ROT) were washed three times in ice cold TEGT. Cytosol was prepared as follows: For Scatchard analysis, tissue and cells were homogenized in 1 volume of buffer. For the other experiments, homogenization was performed in 4 volume of a 2-fold diluted buffer. In addition, the crude homogenate was treated with 1 / 4 volume of 0.5% (w / v) dextran-coated charcoal (DCC) for 10 min on ice before high speed centrifugation. The homogenates were centrifuged at 1,200xg for 10 min at 4℃ to yield a nuclear fractions. The supernatants were further centrifuged at 18,000 xg for 20 min and then after removing the floating lipid, the cytosol was prepared by centrigugation again at 35,000 xg for 3 hrs in a high-speed centrifuge (Beckman J21) or by centrifuging at 108,000 xg for 1 hour at 4 ℃ (Hitachi 70P Ultracentrifuge).

ASSAY PROCEDURES

Nuclear receptor assay

The nuclear exchange assay was adopted the modified method of Saiduddin and Zassenhaus (1977). The assay was initiated by adding 0.5 ml nuclear suspension to 6 glass tubes containing 50 1(1 ng) of moxestrol or E_2 tracer. To 3 of these tubes 50 1 DES (100 ng) had been added and the remaining tubes had received
50 1 TEGT buffer only. After incubating for 1 hour at 37℃ the tubes were quickly cooled on ice and washed 3 times with cold buffer. The resultant pellet was extracted with 3 ml of a toluene-isoamyl alcohol (19 / 1, v / v) mixture and then 2 ml of extractant was transfered to scintillation vials containing liquid scintillator and then counted in a Packard Tri-Carb Scintillation Counter. The counting efficiency was calculated by the method of automatic external standardization.

Cytosol receptor assay

To assay the receptors in small quantity, hydroxyl apatite (HA) method was adopted as previously described (Yoon *et al.*, 1989b). HA was equilibrated with TEGT buffer containing 50 mM KCl and suspended at 0.1 g / ml. After incubation of cytosol with labelled steroids, 1.2 ml of the suspension was added to the cytosol and adsorption allowed to continue for 35 min. The HA was then centrifuged at 1000 xg for 30 min and washed 4 times with TEGT+50 mM KCl. Bound form was extracted 2 times with 1 ml of ethanol and its radioactivity determined after evaporation of the ethanol. As the reference method, we used the DCC adsorption method.

After incubation, 0.25 ml of 0.5% (w/v) DCC was added to the cytosol and the samples were left in ice for 10 min. The samples were then centrifuged at 1000 xg for 10 min and the radioactivity of supernatant was counted.

Cytosol exchange assay

The procedure described by Saiduddin and Zassenhaus (1978) was used to dertermine the total binding sites in the cytosol fraction. Preparation of the cytosol fraction was the same as described earlier except that the TEGT buffer containing 1 mM PMSF and 0.02% NaN_3. The cytosol was treated with DCC (10%, v/v) for 15 min at 4℃ to remove the free steroids and the charcoal was removed by centrifugation at 1,500 xg for 30 min. Aliquots (0.5 ml) of the cytosol were incubated with 30 nM tritiated E_2 with or without DES (200 fold excess amount) for 2 hrs. After incubation a 0.25 ml sample was removed from each tube for determination of available binding sites. To determine the total binding sites the remaining mixture was incubated for a further 18 hrs at 25℃ and then the tubes were kept on ice for 30 min and the amount of bound tracer was assayed by the DCC adsorption method.

OTHER METHODS

Protein determination was performed by the method of Lowry *et al.*, (1958) or by the Bio-Rad kits after precipitation of cytosol aliquots with 10% trichloroacetic acid. Bovine serum albumin was used as a standard.

DNA content was determined by a modified method of West *et al.*, (1985). Scatchard plots were analyzed by linear regression. Curve linear Scatchard plots were analyzed by our computer programmed (Scatchard *et al.*, 1949).

Radioimmunoassays of serum or tissue progesterone (P), testosterone (T), and estradiol (E_2) were performed with the antisera as described previously (Yoon *et al.*, 1987, 1989a).

Statistical analysis of the data obtained from normal distribution was performed by Student's t-test. But the data which was not normally distributed in the samples were performed using the Mann-Whitney U test. All results are expressed as the mean 1 SD, standard deviation unless otherwise stated.

RESULTS

DISTRIBUTION OF ER IN OVARIAN CELLS

The receptor contents of rat ovarian tissue are summarized in Table 1. The present results suggested that the majority of ovarian ER was

Table 1. Distribution of estradiol receptors in rat ovarian tissue

Tissue	Receptor concentration	
	Cytosol (fmol / mg protein)	Nuclear (fmol / mg DNA)
Whole ovary	173.5 ± 10.9	109.6 ± 5.7
Granulosa cell	587.5 ± 67.8	306.2 ± 57.7
Residual ovarian tissues	34.9 ± 8.3	24.4 ± 4.3

* Each experiment was repeated 3 times(values are means ± SEM).

* The bindings of 10 nM-tracers(R2858 for ER) in the presence or absence of cold competitors(200 fold excess DES) were determined after 18 h of inculation at 4℃(cytosol) and 1 h at 37℃(nuclear).

Table 2. Effects of in vivo estradiol administration on moxestrol binding in vitro translocation of receptor estradiol complex to nuclei.

Group	Control	Translocated
Hypophysectomized	48.4 ± 3.2	79.4 ± 5.3[a]
Intact	53.5 ± 8.3	64.7 ± 8.6[a]

Values are average ±SE (triplicate determinations). Rat used in translocation study received 10 μg estradiol in sesame oil (sc) at 1 hour before sacrifice.

[a]Significantly ($p<0.01$) different from mean after translocation.

located in GC layer and only a small amount of ER was detected in the remaining residual ovarian tissue after removal of GC.

Translocation of the receptor-estradiol (RE) complex from cytosol to the nucleus were studied (Table 2). In the hypophysectomized (HPX) rat, a significant ($p<0.01$) amount of the RE complex translocated to the nucleus were detected while only a trend was observed in the intact control rat.

SATURATION BINDING STUDIES IN GRANULOSSA CELLS

Estradiol receptors in GC nuclei were apparently saturated at 4 nmol of R2858 for ER in rat, when assayed using DES as competitor. Scatchard analysis revealed a signle class of ER with an apparent Kd of 3.64, 0.92 nmol / l for ER. The binding capacity of ER in nucleus of granulosa cells of rat ovary was more than 335.2, 116.9 fmol / mg DNA.

LIGAND COMPETITION STUDY; SPECIFICITY

The ovarian receptor exhibits a pattern of specificity for estrogens that is similar to the uterine receptor. Maximum competititon of moxesterol (R2858, E_2 binding to cytosolic receptor) occurred with E_2, DES, ethynyl estradiol and estrone (Table 3). The other hormones examined had little effect on competititon for ER, but they reduce the amount of E_2-binding to receptor.

EFFECTS OF DIHYDROTESTOSTERONE ENANTHATE (DHTE) ON THE OVARIAN RECEPTORS

Previously, Saiduddin and Zassenhaus (1984) have demonstrated that testosterone reduced the weight of ovary and that the action of testosterone may act by blocking of the synthesis of the ER. To confirm this concept, we treated E_2 lmg / ml sesame oil from 24 day age by two day intervals until 30 days. One half of the rats were treated two times DHTE (100 g / 100 l sesame oil) on the 4th week. On 1 day later after the last injection, all animals were killed and the steroid receptor levels in ovarian homogenates were measured and summarized in Table 4.

Table 3. Competition of various steroids for rat granulosa cytosol binding of [3H]-moxestrol to cytosol and nuclear fractions.

Steroids	Displacement ability (%)	
	Cytosol receptor	Nuclei receptor
Methyltrienolone	10.0 ± 0.6	9.7 ± 3.3
Testosterone	7.2 ± 0.9	7.3 ± 3.9
5@ -Dihydrotestosterone	5.6 ± 0.7	16.7 ± 7.9
Androstenedione	27.3 ± 5.4	18.5 ± 7.0
Estradiol	100	100.0
Ethynyl estradiol	87.4 ± 8.6	72.5 ± 3.7
Estrone	84.7 ± 7.9	62.4 ± 9.8
Estriol	57.5 ± 9.1	54.7 ± 9.6
Cortisol	29.4 ± 3.1	11.8 ± 7.1
Progesterone	26.1 ± 7.1	25.7 ± 4.7
Diethylstillbesterol(DES)	100	95.0 ± 4.1

* The rat GC pooled cytosol (1 mg / ml) and nuclei (1 mg DNA) were incubated with 10 nM tracers for 18hr at 4℃ for cytosol and for 1 hr at 37℃ for nuclei fraction in the presence or absence of a 200 fold molar excess of cold steroid. The bound steroids were separated by hydroxyl apatitite. The displacement ability is compared for those in the absence of competitors (0%) and in the presence of a 200 fold excess of unlabeled DES. Values given are the mean of three separate experiments in triplicate.

The cytosolic ER and ovarian wet weight were significantly reduced in rat ovary (Table 4). At this time, 24 hrs later, the follicles became to be atretic by the morphologic criteria. We confirmed again that TR in ovarian homogenates of DHTE treated rats were not significantly changed.

ACID PHOSPHATASE

The percent of positive AcP activity for antral follicle is shown in Table 5. Significant differences were found between the control and PMSG or DHTE treated group but not between PMSG and DHTE group for the number of antral follicles

Table 4. Effects of dihydrotestosterone enanthate on the level of ovarian cytosolic estradiol receptor in rat.

	Treatment		
	Estradiol	DHTE	t-test
ovarian wet weight (mg)	5.3 ± 1.2 (n = 15)	3.3 ± 1.3 (n = 5)	$p < 0.001$
ovarian E_2 receptor	177.8 ± 16.5	122.3 ± 25.1*	$p < 0.01$

* Rats (3 weeks old) were injected 1 mg estradiol / ml sesame oil by 2 days intervals and on the 4th week, rats were injected with 1 mg-dihydrotestosterone enanthate / ml sesame oil. All rats were killed 1 days later after treatment and estradiol receptor levels measured and expressed as bound-steroid (fmol) / mg cytosol protein.

Table 5. Percent of positive reaction for acid phosphatase in frozen sections of antral follicles

Treatment group	n	Acid phosphatase in granulosa cells	
		Grade 1	Grade 2
1. Saline - oil	15	0.2 ± 0.1	21.5 ± 3.8
2. PMSG - oil	12	28.5 ± 6.3	54.1 ± 6.5
3. PMSG - DHTE	18	31.7 ± 4.3	50.1 ± 4.8
4. Saline - DHTE	24	32.3 ± 6.7	52.9 ± 7.7

No significant differences were found between group 2 and 3 but group 2, 3 and 4 were significantly different from control (1) by Mann-Whitney U test ($p<0.001$). The first injection was given at 10:00 AM on day 30 and the second injection of DHTE or vehicle at 4:00 PM on day 31 after 30 hrs later and sacrificed after 24 hrs later.

with positive AcP reactivity. The presence of a positive reaction for AcP aparrently correlated well with localization of the morphological sign of follicular atresia.

HISTOLOGICAL DETERMINATION OF ATRESIA

Table 6 gives the percentages of atresia for the experiments where 1 mg of or 5 mg of DHTE was used. By the treatment of PMSG or PMSG--DHTE, the percentages of atretic follicles were significantly increased in antral follicle (type 5A-8). However, no significant difference in antral follicles between the treated groups were found. By contrast, analysis of the incidence of atresia within in primary follicle (type 3A) showed a significant decrease in DHTE treated group but

Table 6. Percentage of antral atretic follicles in the ovaries treated with dihydrotestosterone enanthate

Treatment group	Dihydrotestosterone enanthate (DHTE)			
	n	DHTE (1mg / kg BW)	n	DHTE (5mg / kg BW)
1. Saline - oil	10	89.3 ± 1.7 (49.1 ± 5.9)	10	85.3 ± 2.7 (54.3 ± 8.8)
2. PMSG - oil	15	89.5 ± 2.9 (52.6 ± 6.7)	13	91.5 ± 3.5 (35.6 ± 6.5)
3. PMSG - DHTE	12	88.7 ± 5.7 (29.9 ± 5.9)	15	91.9 ± 3.7 (21.9 ± 6.6)

Animals were treated with 1 mg DHTE / kg body weight and 5 mg / kg of body weight on day 31 at 4:00 PM. The numerals in the parentheses are the percentages of follicular atresia among total number of follicles including the primary follicles (mean ± SD).

Table 7. Number of follicles classified by the morphological crteria in the ovary of rat treated with pregnant mare's serum gonadotropin (35 IU) or dihydrotestosterone enanthate.

Treatment group	n	Follicle classification		
		Primary (3A) (type 3A)	Preantral (type 4-5)	Antral (type 6-8)
Saline - DHTE	10	69.5 ± 6.4	30.7 ± 3.4	2.1 ± 0.9
PMSG - DHTE				
1 mg	15	68.3 ± 15.3	43.7 ± 7.7	18.3 ± 3.6
2 mg	11	105.9 ± 24.6	32.3 ± 3.7	11.5 ± 2.5
5 mg	10	146.3 ± 39.5	25.6 ± 4.3	8.9 ± 1.2
10 mg	15	268.7 ± 33.7	36.2 ± 4.5	15.3 ± 5.2

Developmental follicular stage was classified by Pedersen and Peters scheme based upon the mouse ovarian follicles. Of the classification, the primary follicles type 3A was the ones with up to 60 granulosa cells; and the preantral group, classes 4 to 5B with up to 400 GCs; The antral follicles, classes 6 to 8 with GCs > 400.

the absolute number of atresia was not changed (Table 7).

PMSG increased serum P levels in all groups. In the DHTE treated group P levels were not significantly different from those achieved with PMSG alone, although it appeared that DHTE had differential effects on PMSG increased P levels.

DISCUSSION

The present study confirmed again that the DCC adsorption assays often gave more variable results due to "stripping" of bound E from the receptor. The DCC adsorption assay was sensitive to the protein concentration (more than 2mg protein) of cytosol. The present study also confirmed that hydroxy apatite method was more reliable and suitable to use in measurement of steroid receptor in ovarian granulosa cells.

Bulk biochemical assays generally do not discriminate among various tissue components. Recently, a variety of histochemical techniques including autoradiography, immunocytochemistry and immunohistofluorescene techniques has been tried to identify receptors in different cell types (DeGoeu *et al.*, 1984; Perrot-Applanat *et al.*, 1985, 1987). Recent papers measuring steroid receptors using monoclonal antibodies describe exclusively a nuclear distribution of receptor observed in cells of reproductive tissuse (King and Greene, 1984; Press and Greene, 1988). The present study also demonstrates that a large number of ER could be determined in the nuclear fractions of granulosa cells.

The concentration of cytosol receptor in the intact rat ovary was Ca 8~10% that found in the uterine (540.3, 36.4 fmol / mg protein). However, the nuclear receptor (25~48 fmol / mg DNA) was present in about equal amount in both organs. Following hypophysectomy the available cytosol receptor (128.4, 19.7 fmol / mg DNA) in the ovary after chopping the internal steroids was significantly higher than that in the intact ovary while no change was observed in the uterus. This may be a reflection of the ER level in the ovary secreted by the ovary itself.

The translocation of the ER complex to nucleus appears to be an obligatory event in the action mechanism of steroids. Our study shows that in the HPX rat ovary a significant amount (Table 2) of ER complex translocated to the nucleus while only a trend was observed in the intact rat ovary. This result demonstrates that the translocation of ER does occur in the ovary

and that the steroids in follicular fluids play a role in the ovary as local regulatory regulators for the follicular cell functions.

Based upon the binding specificity experiments showing the maximum competition achieved with the potent estrogens, DES, ethynyl estradiol, estrone and estriol, the ovarian ER exhibits a pattern of specificity for estrogens. The non-estrogenic steroids did not compete with estradiol. We confirmed that the ER appeared to be labile protein to proteolytic enzymes such as pronase and also quite thermolabile. The heated cytosol at 37℃ for 30 min before assays reduced ER to 25 of specific binding in the ovary.

We confirmed that the treatment of DHTE reduced the ovarian weight and ER significantly. This dose (1 mg/kg body weight) of DHTE increased the percentages of follicular atresia and also the degeneration of preovulatory follicles. Although there was a good correlation between positive acid phosphatase activity and the percent of atretic follicles, the frozen sections did not fix and stain satisfactoryly to provide sufficient resolution of under ordinary microscope to allow assessment of atresia. On the contrary of the result of Kohut *et al.*, (1985), DHTE increased the percentage of follicular atresia in the antral follicles (from type 5A to 8) when treated without any gonadotropins i.e. PMSG. However we confirmed the results of Kohut *et al.*, that DHTE increased the number of the primary follicle (type 3A). Kohut *et al.*, suggested that the percentages of atresia was increased by the treatment of DHT by calculating the percent of atretic follicles among the total mumber of the follicles in the ovary. Farooki and Desjardins (1983) reported that DHT does not increase atresia in some circumstances by evidenced by absence of differences in ovulation rates. These studies treated DHT with PMSG and showed that there was no differences in the percentages of atretic follicle among total number of follicles. However, we observed that the percentages of atresia of antral follicles among the total number of antral follicles were increased by the treatment of DHTE. This result suggests that DHTE induced the atresia only in the antral follicles. This notion could confirmed by the results of Bagnell *et al.*, (1982) and also the fact that the primary follicles does not respond to the gonadotropin and PMSG (Byskov, 1978).

In addition, high dose (>20 IU) of PMSG itself induced the degeneration of ovulated oocytes and also the prematured rupture and luteinization of the follicles in immature rat. Our results demonstrated that there was no difference between the percentage of atretic follicles in the ovary treated with PMSG-oil and that of PMSG-DHTE. This result shows that the treatment of high dose of PMSG or DHTE increases the percentage of atresia but DHTE treatment 30 hrs later after PMSG treat did not enhanced the effects of PMSG on inducing follicular atresia.

In experimentally induced atretic follicles of the hamster there is a shift of steroidogenesis from estradiol to progesterone (Terranova, 1981). Kohut *et al.*, (1985) could not determined that there is no increase in the production of progesterone by the treatment of DHT, but we found the reduction of estradiol level in serum and ovarian veins by the treatment of DHTE alone (data not shown). This result provides further supporting evidence that DHTE induce the follicular atresia. In addition, the present study support this notion by demonstrating that DHTE reduced the amount of ER in the ovary.

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