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Gonadotropin Bioactivity and Steroids in Ovarian Follicle Matured by Hyperstimulation

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과배란유도에 의해 성숙된 여포의 GTH활성도와 스테로이드합성

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〔요 약〕

본 연구는 생식주기중 폐쇄여포액내에서 생물학적, 면역학적 특성을 나타내는 GTH의 변화를 조사하고 steroid hormone과의 상관관계를 조사하며 국부조절인자로서의 GTH의 역활을 조사하고자 하였다.

가임기간중 215개의 여포와 IVF과정에서 185개의 여포를 얻어 여포액내 GTH의 생물학적 또는 면역학적 활성을 측정하였다. Bioactive LH(bLH)는 생쥐의 Leydig cell-testosterone production assay, bFSH는 흰쥐의 Sertoli cell aromatase assay로 측정하였다. Immunological GTH (iLH, iFSH)는 MaiaClone RIA, Delfia kits를 사용하였다.

여포액내 iLH, iFSH, ihCG는 hyperstimulation에 의해 형성된 여포의 크기와는 무관하였다. 또 hMG, huFSH의 처리와도 상관성이 없었다. T의 농도가 높은 여포액내의 iFSH는 현저히 낮았으며 E, P가 고농도인 여포의 ihCG양은 현저히 낮았다. 과배란이 유도된 난소의 여포액내 iLH는 LH specific RIA로 측정시 3mIU/ml이하이었다. 생식주기중 여포액내 bLH, bFSH는 배란기에 현저히 증가 하였다. 혈청내 GTH B/I ratio는 일정한 반면 여포액내 LH, FSH의 생물학적, 면역학적 활성은 미수정란을 가지거나 폐쇄된 여포내의 활성보다 현저하게 높았다.

위의 결과로 보아 여포액내 생식소자극호르몬은 면역학적활성보다 높은 생물학적 활성을 가지며, 생리적 현상의 지표가 된다고 추론된다. 또한 steroid, bGTH는 여포의 선택, 폐쇄를 구분하는 지표로 사용가능하며, 여포가 폐쇄될때 여포액내 B/I ratio가 현저히 낮아지는 것으로 보아 GTH의 활성이 감소되는 것으로 판단된다.

INTRODUCTION

It is common practice in ovarian hyperstimulation for human IVF and ET to monitor the ovarian responses by measuring the concentrations of ovarian steroids in serum. These ovarian steroids are the sum of the production of several ovarian follicles. The measurement of steroid concentrations in the follicular fluid resulted in valuable informations about the optimal steroid environment to select the good quality of oocytes for IVF and embryo growth(Fowler et al., 1978; Wramsby et al., 1981; Car-son et al., 1982; Fishel et al., 1983; Botero-Ruiz et al., 1984; Uehara et al., 1985; Lobo et al., 198; Reinthaller et al., 1987; Yoon et al., 1989).

However, a link between the gonadotropin levels within the follicle and the corresponding

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steroid levels is still missing except our previous study(De Geyter et al., 1986). Previous investigations did not differentiate between intrafollicular LH and hCG (De Geyter et al., 1986; Botero-Ruiz et al., 1984). Knowledge about the real LH concentration in FF is becoming more and more interesting because the roles of gonadotropins(GTH) on ovarian cells are elucidating and also because GTHs are important intragonadal hormones. McNatty et al.(1975, 1985) reported that FF LH concentrations are highest in the largest follicles obtained during the late follicular phase. In the previous study(De Geyter et al., 1986), it was demonstrated that follicular concentration of hCG but not of LH or FSH, serves as an indicator of follicular maturity in IVF programme. Until now there is no enough data on gonadotropins in follicular fluid from spontaneous or hyperstimulated cycles and on their roles in the follicles as intragnoadal regulatory local hormones. In addition, no study has attempted to correlate FF GTH with other hormonal characteristics or with follicular atresia in the spontaneous or stimulated cycles.

Anterior pituitary glands of many mammalian animal species secrete GTHs in multiple heterogeneous forms. That is why the role of GTHs in follicles was difficult to be elucidated. These GTH isohormones exhibit different molecular weights, isoelectric properties, bioactivities, and plasma half-life etc.(Khan et al., 1984; Harlin et al., 1986; Wang et al., 1988; Nomura et al., 1989). Most of the physiological and clinical studies on serum or urine gonadotropins had been measured by specific radioimmunoassays. Recent studies showed that changes of biological activities of GTHs are not always associated with similar changes in GTH immunoactivities (Khan et al., 1984; Wang et al., 1988).

The present study investigates the bioactivity and immunoactivity of FSH, LH and hCG in ovarian follicles from stimulated either with hMF or with huFSH or from spontaneous cycles in order to study the role of GTHs on follicle maturation and atresia. Comparisons are made with the follicular concentrations of E, P, T and DHT and also with the immunoactivity of GTH.

MATERIALS AND METHODS

Human follicles(n=215) from spontaneous cycles were obtained from 86 patients at the random. phase of menstrual cycle, immediately after surgery from the three local hospitals. The indications of surgery were as follows;10 with pelvic inflammatory disease(mean age 41.0±7.1), 23 with uterine myoma(33.7±8.7), and the others with unknown disease(38.0±7.5), mainly during hysterectomy.

185 follicular fluid samples were collected in 63 cycles from 41 women treated for IVF and ET because of tubal blockage. The mean age of the patients was 34 years(range;27 to 46). Ovarian hyperstimulation was performed by administrating either hMG(Humegon, Organon) or huFSH(Fertinorm, Serono) as described in the previous paper(De Geyter et al., 1986). The ovarian stimulation was monitored by daily measurements of E, P and LH in serum. Ovulation was induced as intramuscular injection of 10,000IU hCG(pregnyl, Organon). At 34 to 36 hours after the hCG injection a laparoscopy was carried out to aspirate the content of the follicle. After centrifuging at 800g for 10min at 4 ℃, the fluid was stored at -20℃.

bLH was measured using the modified testosterone production assay(TPA) with mouse Leydig cell described by Van Damme et al.(1974). All samples were measured in triplicate, at dilution of 1/10, 1/20 and 1/40 by incubation with a crude cell preparation of mouse Leydig cells (150,000/tube) for 3-4hrs at 32℃ under a 95% air:5% CO_2 mixture. The resulting medium was assayed for testoterone using the established LIA(Yoon et al., 1987). The limit of sensitivity of the assay using 1/10 dilution was 0.2ng LER 907. Both inter- and intra-assay coefficient of variation were<10%. Especially for the samples of hyperstimulated cycles, whose LH concentrations are very low, we used an in vitro assay, determining cAMP accumulation in cultured rat Leydig cells. The cells were prepared and suspended in Ham's F-10 medium with 10 % fetal calf serum, and aliquots of cells(100,

000/well) were plated in 24-well dishes(A/S Nunc,) followed by incubation at 37℃ in 5% air. The medium was removed 18hr later, and the cells were incubated in Ham's F-10 with 0.1 % BSA for an additional 2hr. The medium was then replaced by Ham's F-10 with 0.1% BSA containing 4mM theophylline and samples(2-10 ul) and standards(1-1,000ng/ml). After 1hr incubation with sample, dishes were immersed in boiling water for 5min to stop the reaction. cAMP was measured using commercial RIA kits of New England Nuclear Corp.

For in vitro FSH bioassay using Sertoli cell aromatase system, 48 testes from 7 to 10 day-old-Sprague-Dawley rats were decapsulated and subjected to a two-step collagenase dispersion. After the initial dispersal, the seminiferous tubules were settled to the bottom of incubation flask and then upper portions of Leydig cells were removed. During the second dispersion, the Sertoli cells were dissociated from the tubules as follows:The tubules were washed severnal times with medium containing collagenase 0.03 %(w/v), soybean trypsin inhibitor) 0.003%(w/v) and DNase 0.03% (to prevent clumping of cells) and then incubated for 30min at 34℃. During incubation, dispersion of Sertoli cell was hastened by repeated aspiration with a Pasteur pipettes. The resulting cell suspension was washed three times. The cells were washed to remove enzymes and plated at a final density of approximately 500,000 cells/ml medium. Viability and the number of the cells were determined by tryphan blue dye exclusion tests. The culture medium consisted of a mixture of Ham's F-10 nutrient mixture and Dulbecco's Modified Eagle's medium(1:1, v/v) supplemented with 1.2g/l sodium bicarbonate, 20mg/l gentamycin and 1mg/l amphoterin, 1μg/ml insulin, 5μg/ml transferrin, 10ng/ml epidermal growth factor, 20pg/T4, 100μM hydrocortison and 1μM retinoic acid, 0.1mmol/l methylisobutylxanthine, and 2μmol/l 19-hydroxyandrostenedione as a substrate. After a 72hrs incubation, the cell monolayers were washed once, then reincubated in fresh medium containing concentrations of hFSH-3 or serum or FF samples in triplicates(5 to 20ul), and the incubation was continued for an additional 24h. The medium was then aspirated and centrifuged, and the supernatant was frozen for E measurement. The medium E concentrations were determined in duplicates.

Laboratory details for ovum cultures are described elsewhere(Sololoski and wolf, 1984). The quality of the culture system was regularly checked with mouse ova and embryo quality control system. Healthy follicles were distinguished from atretic ones on the bases of the following criteria: high percentages(5-10cells/ml) of pyknotic index and the number of granulosa cells;increase of GC nucleus diameter, morphological aspect of the oocytes such as degeneration, fragmentation or second polar body extrusion;oil drops and color of follicular fluid; P and T dominant-E depleted conditions in FF etc (Bomsel-Helmreich et al., 1979).

Immunoactivity of FSH was measured with a commercial kit (Delphia hFSH, LKB). Follicular LH was measured with LH MaiaClone (serono) and follicular hCG with hCG MaiaClone(Serono). When the cross reactivity for each of these assays was rechecked in our laboratory, we found that the LH MaiaClone did not have any detectable cross reactivity with hCG. In the presence of 1,000mU/ml hCG in serum and follicular fluid, the LH MaiaClone assay did not measured LH activity. The lowest limit of detection for FSH was 1mU/ml, for LH 0.1mU/ml, for hCG 1mU/ml. E was measured with a commercial kit(Radio Isotopen Service, Wuerenlingen, Switzerland). P was measured with a commercial kit(Biodata, Rome, Italy). T and DHT were measured with the same antibody(Yoon and Kim, 1987; Yoon et al., 1987) after separation with HPLC. The intraassay coefficient of variation was below 11.2% in the gonadotropin assays and below 13.2% in the steroid assays. The interassay coefficient of variation was below 11.2% in the gonadotropin assays and below 15.6% in the steroid assays.

The fertilization rate(%) is defined as the number of fertilized oocytes divided by that of inseminated oocytes. The cleavage rate of cultured embryos is defined by the number of

blastomeres per embryo after a constant period of growth. Oocyte fertilization rate(OFR) and embryo cleavage rate(ECR) are compared using the Chi square tests. Follicular volume is analysed using the Mann-Whitney U test and hormone concentrations are analysed with Student's T test after logarithmic transformation. The hormone concentrations are presented by the geometrical mean with the standard deviation(SD).

RESULTS

Using the recently launched and commercially available IRMA kits for serum iLH(MaiaClone), which are claimed to specifically measure serum iLH in the presence of hCG, the possibilities that the specific LH or FSH in serum and in FF were tested. For the measurements of GTHs and hCG in serum, three assay methods including WHORIA, were compared, showing good correlations($r>0.88$). However, to determine the GTH levels in FF from hyperstimulated cycles, Delfia and WHORIA were not suitable because of the higher cross reactivities with hCG. The specificity of the LH MaiaClone assay system was assessed by measuring the apparent LH response caused by high level of hCG (1,000IU/ml, in terms of hCG 1st IRP 75/537). The cross reactivity with hCG in LH MaiaClone and that with LH in hCG MaiaClone assay were <0.003 % and <0.97% respectively.

The concentrations of iLH, iFSH, ihCG and iLH/iFSH do not vary according to the aspirate volume of the follicle as shown in the Table 1.

Of the 138 follicles, 68 samples were obtained from the cycle stimulated with hMG and 75 with huFSH. Of the 63 oocytes 35 ova were originated from follicles treated with hMG and 28 with huFSH. The fertilization rate of huFSH treated group was 68% and that of hMG stimu-

Table 1. Concentration of LH, FSH, hCG and LH/FSH ratio in the follicular fluid obtained from the hyperstimulated cycle

Aspirate volume:	LH (mU/ml)	FSH (mU/ml)	LH/FSH	hCG (mU/ml)
<1ml	2.76±1.12	3.30±1.41	1.04±0.65	7.53±18.95
3~4ml	3.11±1.97	3.28±1.47	1.09±0.81	121.89±76.96
6ml	2.00±1.03	2.83±1.14	0.86±0.52	67.20±42.36

Follicles were grouped into 3 classes according to the volume of the aspirates. The values are expressed as the means±SD. No significant differences are detected.

Table 2. Comparison between the concentrations of gonadotropins and steroid hormones in follicular fluid from hMG treated cycles and those from huFSH treated cycles

Characters	hMG	huFSH
Aspirate volume (ml)	2.96±1.93	2.53±2.19
LH (mU/ml)	3.23±2.05	2.73±1.20
FSH (mU/ml)	2.89±1.31	3.17±1.17
LH/FSH	1.30±0.89	0.99±1.10
hCG (mU/ml)	115.91±81.4	94.31±46.25
Estradiol (nmol/l)	1687.3±926.1	1282.5±692.3*
Progesterone (umol/l)	13.94±9.85	14.53±10.65
Testosterone (nmol/l)	6.38±4.07	7.21±4.84
DHT (nmol/l)	1.82±0.80	2.08±0.79

The data are grouped according to medication used for ovarian hyperstimulation: 68 follicles are stimulated with hMG, 75 with huFSH. The figures represent the mean values±SD. * indicates a significant difference of $P<0.05$.

lation was 83%. This difference was not statistically significant. There were no differences in the embryo cleavage rates after both stimulation regimens. In Table 2 the effect of the medication used for ovarian hyperstimulation on the hormonal levels in the follicular fluid is shown. No statistically significant differences in the FF gonadotropin concentrations were detected. HMG treatment results in a significantly higher follicular E concentration compared to huFSH stimulation($p<0.05$). The aspirates by hMG treatment have a slightly larger volume of FF($p<0.05$).

Grouping the follicles according to the concentrations of E,P, T. and DHT to show the relationships between the levels of steroid hormo-

Table 3. Concentrations of gonadotropins in follicular fluid grouped according to the concentrations of steroid hormones in the follicles

Steroids	LH (mU/ml)	FSH (mU/ml)	LH/FSH ratis	hCG (mU/ml)
Estradiol				
>1400 nmol/1:	3.20±1.46	3.08±1.33	1.21±0.61	113.27±74.86
<1400 nmol/1:	2.94±1.63	3.10±1.16	1.12±0.46	100.94±56.65
Progesterone				
>10 umol/1:	3.12±1.44	3.12±1.31	2.20±0.68	95.56±59.50
<10 umol/1:	3.01±1.67	3.09±1.17	1.13±0.72	113.25±65.90
Estradiol				
>1400 nmol/1: and progesterone	3.15±1.39	3.07±1.23	1.19±0.61	75.27±23.78**
>10 umol/1: remaining follicle	3.03±1.61	3.10±1.23	1.15±0.72	111.90±64.7**
Testosterone				
<10 nmol/1:	3.08±1.56	3.10±1.52*	1.15±0.44	107.23±73.60
>10 nmol/1:	2.62±1.27	2.40±0.98*	1.24±0.98	102.93±62.65
Dihydrotestosterone				
<2.35 nmol/1:	3.12±1.63	2.78±1.13	1.23±0.70	110.70±74.15
>2.35 nmol/1:	2.86±1.46	3.22±1.35	1.05±0.60	92.35±54.65

The follicles are grouped according to the concentrations of E, P, T and DHT. The comparison is made with the follicular concentrations of LH, FSH, LH/FSH and hCG. The values represent the mean±the standard deviation. * indicates a significant difference of $P<0.05$ and * * indicates a significant difference of $P<0.01$.

Table 4. Comparison of immunoreactive gonadotropin concentrations determined by different assay methods in the follicles with fertilized ova and those with unfertilized ova

Hormones	Fertilized	Unfertilized
Luteinizing hormone (1 st IRP, 68/40)		
WHORIA	32.78± 2.17 (13)	40.69±18.27 (23)
Delfia	48.33±41.06 (18)	68.11±40.53 (18)
MaiaClone	2.99± 2.14 (19)**	2.23± 1.35 (16)**
Follicle Stimulating Hormone (2nd IRP, 78/549)		
Whoria	12.11±0.73 (50)	6.32±0.45 (22)
Delfia	2.53±2.33 (18)	2.22±0.83 (21)
Human Chronic Gonadotropin (1st IRP 80/558)		
Delfia	133.76± 8.65 (12)	108.78±10.64 (11)
MaiaClone	84.11±66.49 (18)	174.03±86.71 (10)

The numerals in the parentheses are the number of the tested samples. The values represent the mean ±standard deviation. The concentration of LH were calculated in terms of 1 st IRP, 68/40 and that of FSH in terms of 2 nd IRP, 78/549, and that of hCG, 1 st IRP 80/558.

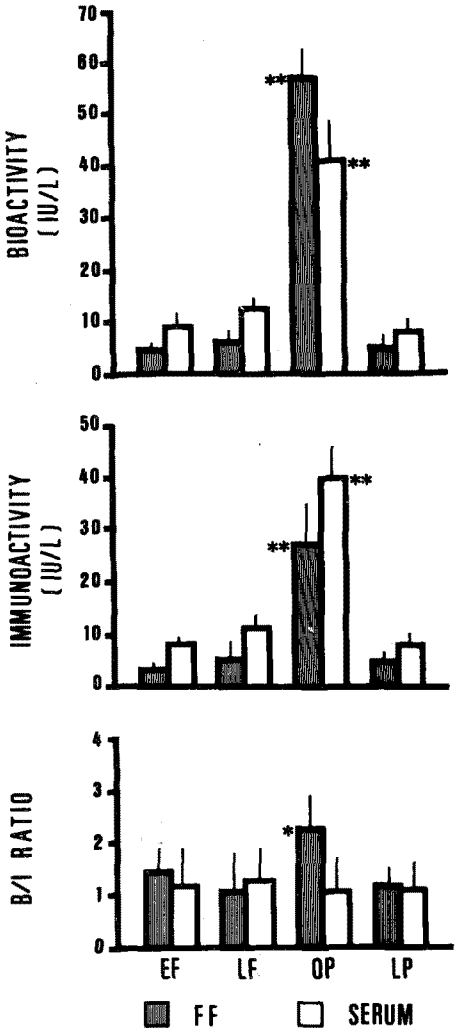

Fig. 1. Biological and immunological activities of LH in antral fluid of ovarian normal follicle from the spontaneous cycle.

Bioactivity of LH was determined by TPA using mouse Leydig cells and in terms of LH 69/104. Immunoactive LH was determined by MaiaClone LH kits and expressed in terms of LH 68/40. 115 normal follicles(size>8mm) in follicular phase were obtained and grouped into the early(EF), mid and late(LF) follicular phase, ovulatory period(OP:iLH>30 U/L in serum by MaiaClone kits), and 45(size>6mm) follicles were grouped into luteal phase(LP). The column with thin lines show the values in follicular fluids(FF) and that with open columns are the values in serum. *indicates $p<0.05$ and **$p<0.01$.

nes and GTH concentrations, Table 3 shows the levels of GTHs and their ratios in FF from hyperstimulated cycles. The mean concentrations of iFSH are significantly lower in the follicles characterized by a higher amount of T concentrations. The level of hCG is significantly lower

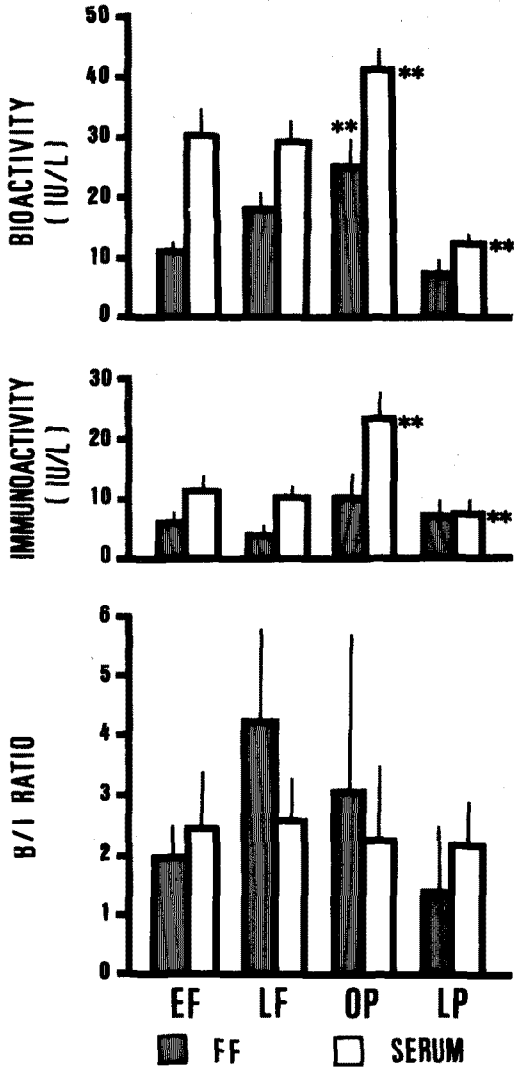

Fig. 2. Biological and immunological activites of FSH in antral fluid of ovarian normal follicles from the spontaneous cycle.

Bioactivity of FSH was determined using Sertoli cell aromatase bioassay with immature rats and expressed as the potency of WHO urinary FSH/LH 70/45. Legends for the follicle grouping and for the descriptions are same as Fig. 1. Values are the means of three triplicates. iFSH levels are shown in terms of 2nd IRP, 78/549. * indicates $p<0.05$;**$p<0.01$(EF vs OP or LP).

in the follicles with higher E and P concentrations($p<0.01$).

The immunoreactivities of iLH, iFSH, and ihCG were measured in FF from hyperstimulated follicles and grouped into two according to the fertilizability of the oocyes(Table 4).

The levels of iLH measured by MaiaClone were significantly lower than those measured by WHORIA or Delfia. This result shows that there are only a little iLH in FF but a huge amount of ihCG in FF from hyperstimulated cycle.

The biological and immunological activities of GTHs in serum and follicular fluid from the spontaneous cycles were summarized in Fig. 1 (LH) and 2(FSH).

Both activities of LH were increased in the serum and in FF during the ovulatory period of the menstrual cycles but the B/I ratio was rather consistant during the cycles(Fig. 1). Both activities of FSH were increased during the ovulatory period in the spontaneous cycles. The bioactivity of FSH(bFSH) in follicular fluid was significantly increased during this period but that in serum was not changed in the menstrual cycles(Fig. 2).

The biological and immunological activities of GTHs in the follicular fluid from hyperstimulated cycles were grouped according to the fertilizability of the oocytes cultured in vitro (Fig. 3).

bLH and bFSH in Ff of the fertilized oocytes were significantly higher than those in the FF of unfertilized group and in the atertic follicular fluid whose oocytes were degenerated or fragmented during the culture period and not fertilized.

DISCUSSION

When we have used zero dose sera and the sera containing 1,000IU/ml(1st WHO IRP-hCG 75/589) in the MaiaClone assay system, iLH levels in sera was not changed. This result demonstrates that the cross reactivity of MaiaClone monoclonal antibody with LH was very low and that the iLH determined by MaiaClone in FF from hyperstimulated cycles is specific LH.

Medications for ovarian hyperstimulation allowing the regulations of LH or FSH levels, and LH/FSH ratio during the treatment (e.g. huFSH, GnRH agonists) have recently been introduced (Veeck, 1986). The usefulness of these medications has to be confirmed in two ways. First we wanted to determine whether two different medications, hMG or huFSH, can modify the

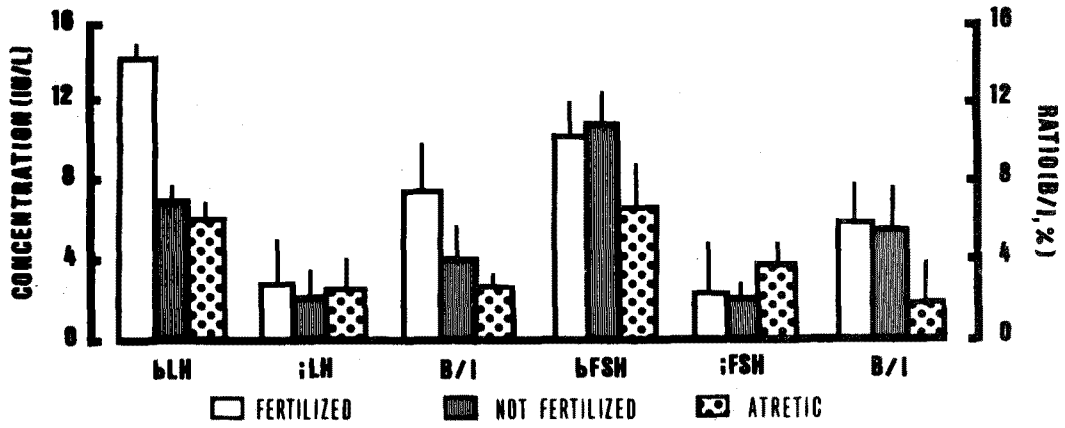

Fig. 3. Biological and immunological activities of gonadotropins in follicular fluids of the preovulatory follicles in the hyperstimulated ovary.

bLH or bFSH represents the biological activities and iLH, iFSH depicts the immunological activity. Their ratios(B/I) were calculated. The bLH were determined in the follicular fluids after hCG in FF were absorved by the hCG antibody. The follicles were grouped based upon the fertilizability of the oocytes, pyknosis of granulosa cells and the contents of steroids in FF. *indicates $p<0.05$;**$p<0.01$ (fertilized vs atresia or unfertilized).

hormonal environment in the ovary. Secondly, it was also important to demonstrate that differences in the follicular gonadotropin concentrations correlate with differences in the quality of the oocytes, originating from these follicles. We found that iLH in the follicular fluid from huFSH stimulation, results only from the endogenous secretion, in contrast to hMG treatment, which adds exogenous LH activity. Nevertheless, hMG stimulation causes only a slight increase in the follicular LH concentration and LH/FSH ratio. However, whether these minor differences in the follicular LH and FSH concentrations are accompanied by significantly higher follicular estradiol concentration after hMG treatment or not is to be elucidated. The fact that the follicular concentration of E is higher after hMG stimulation to compared to huFSH stimulation is in agreement with previous finding by Martikainen et al.(1986). Polan et al.(1986) found similar concentrations in the FF after both treatment regimens. The good correlation between the follicular E and P with the quality of the oocyte confirms previous findings(Fowler et al., 1978;Wramsby et al., 1981; Carson et al., 1982). It justifies the use of the cleavage rate, as defined in this study, to assess the quality of the oocytes.

Differences in the concentrations of LH and hCG and in the LH/FSH ratio in the antral fluids of the preovulatory follicle are correlated with differences in oocyte maturity(De Geyter et al., 1986). The present study also indicates that oocyte quality can be assessed by the bioactive gonadotropin levels in the follicular fluid. Informations about the most appropriate bGTH cocentration for follicle quality will be helpful to develop criteria for ovarian stimulation regimens adapted to the individual needs of the patients.

Veeck(1986) and Scoccia et al.(1987) noted the disparity between the morphological status of the oocyte-cumulus complex and the nuclear maturation of the oocyte after huFSH stimulation. According to Eppig(1979), FSH determines the morphology of the oocyte-cumulus complexes. Our study does not prove this notion. Whether the nonsignificant slight increase in follicular FSH concentration, induced by the huFSH stimulation, is responsible for this effect or not is to be cleared. The use of varying protocols for ovarian hyperstimulation makes an objective assessment of the oocyte's maturity mandatory(Veeck, 1986; Scoccia et al., 1987). The E and P concentrations in the follicular fluid have been evaluated for this purpose(Fowler et al., 1978; Wramsby et al., 1981; Ca-rson et al., 1982; Botero-Ruiz et al., 1984; Re-inthaller et al., 1987). Our results indicate that E concentration in the follicular fluid is depen-dent on the treatment chosen. It is also known that aromatase activity is suppressed by high concentrations of hCG or LH(Moror 1974;Ainsworth et al., 1980). E concentration together with P concentration may not be suitable as a marker for follicular maturity because of its dependence on variabilities other than follicular maturity. The follicular concentration of hCG is inversely correlated with the cleavage rate of the embryo. This is in disaggrement with previous observations by Laufer et al.(1984). The speculation that follicular atresia is responsible for the reduction in the quality of the embryos in presence of high concentrations of hCG can be ruled out(Richards, 1980). The T concentration is not significantly elevated in follicles with high levels of LH and hCG.

Injection dose, diffusion time and metabolic clearance rates being constant, the final concentration of hCG in the follicular fluid is only dependent on the permeability of the follicle wall. The permeability of the follicle wall is known to increase during follicular maturation (Zachariae 1958). Consequently, hCG concentration in the follicular fluid reflects the maturity state of the follicle. The parallels between hCG and LH levels in the follicular fluid support this hypothesis(De Geyter et al., 1986).

To find out the criteria for the oocyte maturation and for the follicular atresia, the concentrations of steriod hormones and GTHs in FF have been evaluated(Fowler et al., 1978; Wramosby et al., 1981;Carson et al., 1982; Botero-Ruiz, 1984;Reinthaller et al., 1987). The

present results indicates that E concentration in FF is dependent on the chosen treatment. It is also known that the reduced aromatase activity is the marker of atresia(Ainsworth et al., 1980; Richards, 1980). However the present studies shows that the concentrations of androgens such as T or DHT could not be the marker of atresia, but the reduced B/I ratio could be used as the sign of atresia.

The discrepancies in potency estimates of these GTHs obtained by different methods or in different laboratories have been suggested to be due to several factors such as :1) the heterogeneity known to be associated with GTHs and the emerging evidences that the same hormones may exist in different molecular forms in the test samples in different endocrinological conditions, 2) the influences of reference preparation used as standard in the assays, and 3) the other variables in the RIA systems like the purity of the tracer, the specificity of the antisera and the conditions of incubation(Zaidi et al., 1982; Khan et al., 1984; Harlin et al., 1986; Wang, 1988).

Romani et al.(1977) did not observe qualitative differences between bLH and iLH in plasma samples throughout menstrual cycle, but reported elevated B/I ratios(0.5 using hCG RIA system and 6.4 using LH RIA using the 2nd IRP hMG and the 69/104 IRP). However, Dufau et al.(1974) did not observe a major departure from unity of B/I ratios throughout the menstrual cycle. The present study demonstrates that consistent B/I ratios during the cycle and could not showed any decrease for the day of LH surge day and ovualatory days in menstrual cycle. The B/I ratio in FF seems to be increased during the ovulatory period but was not statistically significient.

Padmanabhan et al.(1988) reported that B/I ratios are increased at the time of the midcycle LH surge day by a factor of 4 times (6.2 ± 1.4) and then decreased during the luteal phase. The present result also shows the increase during the ovulatory phase in FF and sera from spontaneous cycle. The ratio levels of the present study was consistent with the results of Jia et al.(1986).

The serum B/I ratios of LH during the spontaneous cycle has been reported more than 5 during the period when ovarian follicles reached 18mm, compared with 2 or 3 in the early follicular phase(Cha et al., 1986). Our data does not concur with this finding. On the other hands, Cha et al. reported that the B/I ratios in FF were more than 25 in the follicles whose oocytes were immature or could not be obtained and that in the follicle whose oocytes were intermediate or matured, B/I ratios were more than 45. However we never could observe this kind of high B/I ratio in FF throughout the cycle even in the sponttaneous or in the hyperstimulated cycles. This kind of higher ratio could be come from the used system to determine the bLH. They did not stripped the steriod hormones in the follicular fluid and only they inactivated the FF by heat and diluted to 1/160 or 1/640. As shown in the reports and in our previous report, there are a huge amount of steroids in FF(ex more than 12μg/ml P and more than 1μg/ml E in the follicle with immature oocytes and with matured oocytes. Therefore the dilution could not removed the precursors of testosterone, even though they reported that there is only $<$3pg/tube of testosterone, because there will be 10ng/ml P at the dilution of 1,000. Otherwise, the results might be come from the standard used, we could not find the standard characteristics in the report.

Our present study demonstrate very immportant informations that bLH and bFSH in follicular fluid of normally fertilized in IVF/ET programme are significantly higher than those in the FF of atretic follicle and in FF of unfertilized oocyte. Especially the concentration of iLH in the atretic follicles does not different with that in the follicles of normal fertilization but the B/I ratio was significaltly reduced in the atretic follicles. Our data suggest that GTH molecules might be lost their biological activities and molecular compositions might be changed to inactive form. The present study also demonstrated that the B/I ratio could be used as one of the criteria to judge the follicular atresia.

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