The clinical significance of reproductive hormones concentration in follicular maturation in patient with Luteal phase defect

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الخلاصـــــة:

أربعين مريضه أشتركت في هذه الدراسه.عشرين مريضه تعاني من ضعف الجسم الاصفر بينما العشرين الأخريات يتمتعن بدوره طبيعيه وسبب العقم هو أنسداد الأنابيب الرحميه .تم تشخيص المريضات بواسطة قياس الهرمونات وخصوصا هرمون البروجستيرون في اليوم الحادي والعشرين من الدوره فكانت قلة الهرمون في هذا اليوم أقل من 10نانوغرام لكل مللتر تعني وجود ضعف الجسم الأصفر.

كان الهدف من الدراسه:

- قياس تراكيز الهرمونات التناسليه (الهرمون المنشط للجريب ،الهرمون اللوتيني ،الهرمون اللبني ،هرمون البروجستيرون وكذلك هرمون الأستيروجين) في السائل الحويصلي المبيضي للبيوضات الناضجه وغير الناضجه ووسطية النضوج .
- مقارنة معدلات لأخصاب الخارجي في الأنابيب لهذه المجاميع الثلاثه من البيوض ودراسة دور هذه الهرمونات في عمليات الأخصاب الخارجي.

تم سحب السائل الحويصلي وتصنيفه حسب نوع البويضه المستخرجه منه الى ثلاثة مجاميع (ناضجه، وسطية النضوج ، غير ناضجه) ثم تم قياس مستوى الهرمونات باستخدام جهاز mini vidas وتبين أن تراكيز كل من الهرمون المنشط للجريب ، الهرمون اللوتيني ، الهرمون اللبني) كانت عاليه جدا في البيوضات غير الناضجه وبدلاله أحصائيه معنويه($P<0.05$) بينما كانت تراكيز كل من هرمون البروجستيرون والأستروجين عا ليه في البيوضات الناضجه وبدلاله أحصائيه معنويه $P<0.05$. لقد أعطت مجموعة البيوض الناضجه ومتوسطة النضوج معدلات أخصاب عاليه مقارنة بالبيوض غير الناضجه وبدلاله أحصائيه معنويه ($P<0.05$) .

Abstract :

Forty infertile female patients were involved in this study. Twenty infertile female were with luteal phase defect and the rest twenty female were with out luteal phase defect they represent control group .All patient were stimulated with clomiphene citrate (clomid) and human menopausal gonadotropin (Humegon).

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The objectives of the study were:

1-To measure the concentration of follicle stimulating hormone, Luteinizing hormone, prolactine hormone, progesterone and estrogen

hormones in follicular fluid of mature, intermediet and immature oocytes.

2-To compare the in vitro fertilization rate of the three groups of the oocytes.

The luteal phase is characterized by the production of progesterone from the corpus luteum with in the ovary .The corpus luteum is derived both from the granulosa cells that remain after ovulation, and from some of the theca cells which

differentiate to become theca lute in cells. Progesterone produced by the corpus luteum is the dominant hormone of the luteal phase. Serum progesterone level less than 10 ng/ml in cycle day twenty-one indicate the presence of luteal phase defect.

Follicular fluid sample following aspiration of oocytes were classified into three group (mature, intermediate and immature oocytes) depending on the morphological structure of the oocytes and the surrounding cells. The concentrations of follicular fluid hormones were measured by enzymes immuno assay method using the minividas.

Patients with luteal phase defect have significant higher levels of follicle stimulating hormone, Luteinizing hormone and prolactine hormone than patients with out luteal phase defect (control group).

The control group has significant higher level of progesterone and estrogen hormones than luteal phase defect patients. The luteal phase defect patients have the lowest maturation rate and the lowest fertilization rate than control groups.

The mature oocytes gave significant higher concentration of progesterone and estrogen hormones than intermediate or immature oocytes with better in vitro fertilization rate and cleavage rate than the immature oocytes.

The results of the present study indicate that increased levels of gonadotropin in patients suffering from luteal phase defect have a significant clinical importance in the maturation it could lead to inhiption of the growth of oocytes and result in decrement of the maturation rate and this support the threshold theory of gonadotropin.

Those hormones could be considered as potential markers of human oocytes quality and in vitro fertilization.

Introduction:

Fertilization result in human assisted reproduction are influenced by a combination of male and female factors 1.Thus, fertilization failure in an in vitro fertilization attempt can be due to a sperm abnormality, poor oocyte quality or both 2.

During the final phase of ovarian follicular development, the oocyte resides in an antral follicle where it' s initially associated with specialized granulosa cells (cumulus oophorus and corona radiata cells) 3, and where it is exposed to a particular humeral microenvironment (follicular fluid) whose composition differs from that of blood plasma4. The phase of oocyte meiotic and cytoplasmic maturation coinciding with the development and growth antral follicle is subject to a complex interplay of endocrine, paracrine and autocrine control mechanism 5.Hormone and other regulatory substances involved in these mechanism are either locally secreted with in the ovary (steroid hormone, cytokines) or are produced outside and enter the follicles secondarily6. The intrafollicular concentration of some of these agents at specific times of antral follicle development is likely to be related to the success or failure of various development process in the oocyte that are necessary for its fertilizability and further developmental competence 7.

Materials and methods:

1-Patients: -This study involves 40 infertile couples, twenty with luteal phase defect and other twenty represent control group .All the patients attend the in vitro fertilization Institute for embryo research and infertility treatment in Baghdad. Their age range from 20-35 years . The duration of the infertility of these females range from three to ten years. Diagnosis of luteal phase defect depends on measuring serum progesterone level in cycle day 21 serum progesterone less than 10 ng/ml indicate the presence of luteal phase defect.

2-Ovarian stimulation, follicular fluid sampling and oocyte collection:

Ovarian stimulation was induced by Clomiphen citrate (clomid, Merrel company, England) 100mg/day starting from cycle day 2 and for 5 days. The human menopausal gonadotropin (pergonal 75 Iu FSH, 75 Iu LH, per ampoule, Serono company, Italy)was given in cycle day seven the dose (150-300 Iu/day)depending on the ovarian response, levels of serum E2 and ultra sound. When the follicle size reached to >17 mm, and the E2 was 200-250 pg/ml per mature follicle, the patients were injected intramuscularly by 10,000 Iu of human chorionic gonadotropin(pregnyl, Serono company, Italy) in order to induce final maturation of ovarian follicle.

Follicular fluid was sampled by transvaginal ultra sound guided puncture and aspiration of follicles. Each follicle was aspirated separately and collected in a different dish. Fluid aspirated from each individual follicle was maintained isolated from that coming from other

follicle. The characteristics of the aspirated follicle were reported including size of the follicle, wall appearance and the amount and color of the follicular fluid.

3-Determenation of hormones concentrations:-

Enzymes link fluorescent assay technique were used to determined follicular fluid concentrations of estradiol(E2), progesterone(P), follicle stimulating hormone(FSH), Luteinizing hormone(LH) and Prolactin hormone(PRL). The assay principle combines an enzyme immuno assay sandwich method with a final fluorescent detection.

Statistical analysis

Follicular fluid concentration of individual hormones associated with each maturation and viability status of oocytes, with each fertilization result are expressed as mean ± SD. Data were analyzed by using appropriate statistical tests like T-test and chi-squre test were used according to the nature of the data.

Results:-

The mean concentration of FSH hormone (mlu/ml) in the follicular fluid of the immature oocyte was significantly higher than mature oocyte ($P<0.05$). The difference in the mean concentration of follicle stimulating hormone between the intermediate and immature oocyte groups was non significant ($P>0.05$) table (1).

The mean concentration of Luteinizing hormone (mIu/ml) in the follicular fluid of immature oocyte was significantly higher than mature oocyte ($P<0.05$), while no significant differ with intermediate oocyte table (2).

The mean concentration of Prolactin hormone (ng/ml) in the follicular fluid of immature oocytes were significantly higher than mature oocytes ($P<0.05$), while no significant differ with intermediate oocyte ($P>0.05$) table (3).

The mean concentration of estradiol hormone (pg/ml) in the follicular fluid of mature oocytes were significantly higher than immature and intermediate oocytes ($P<0.05$) table (4).

The mean concentrations of progesterone hormone (ng/ml) in the follicular fluid of mature oocytes were significantly higher than immature and intermediate oocyte ($P<0.05$) table (5).

The percentages of fertilization in mature oocytes were significantly higher than intermediate or immature oocytes ($P<0.05$) table (6), it is

also significantly higher in intermediate than immature oocytes ($P<0.05$).

In general the control group give a significantly higher percentage of mature oocyte ($P<0.05$) than patients with luteal phase defect, table (7).

Luteal phase defect patients have the lowest maturation rate and lowest fertilization rate

Discussion:

After the extensive application of in vitro fertilization in the treatment of human infertility in the early 1980 s 8, many studies try to relate hormone and cytokine concentration in follicular fluid to oocyte maturity and fertilization results 9. Some studies shows that there is no relationship between concentration of FSH and LH or the FSH/LH ratio on the one hand, and oocytes meiotic maturity on the other hand 10. Other studies suggest that high LH and FSH production are important for some intra follicular events necessary for optimal cytoplasmic maturation of oocytes 11. Brown suggests that ovarian response is evoked when an FSH serum threshold is reached 12. Further more hillier proposed that in addition to the threshold level of FSH, the follicle has a finite requirement for stimulation by LH which enhance steroidogenesis13, beyond this level, that is to say high level, LH suppresses aromatase activity and inhibit cell growth 14.

In this study the concentration of LH and FSH were significantly higher ($P<0.05$) in the follicular fluid of immature oocytes than mature oocytes.

Oestradiol concentrations in follicular fluid are known to increase before the pre-ovulatory LH surge15, where's the period after the LH surge is characterized by a dramatic increase in the concentration of progesterone 16.

Published data about the relationship between oestradiol and progesterone in human follicular fluid and oocyte maturity and fertilizability are not consistent 17. High oestradiol concentrations were reported to be associated with oocyte maturity 18. Also high concentrations were in follicular fluid of mature oocyte that fertilizes 19. Where's other studies did not find any relationship between estradiol and progesterone concentrations in follicular fluid, on the one hand, and fertilization on the other hand 20.

In this study the concentration of progesterone and estrogens hormones were found to be significantly higher in the follicular fluid of mature oocyte that fertilize.

Some other studies shows that prolactine can suppress aromatase activity of granulosa cells and this support the concept that prolactine may suppress follicular maturation 21.

In this study prolactine concentration was found to be significantly higher in the follicular fluid of immature oocytes.

CONCLUSION:

Mature follicles have a significantly higher concentration of progesterone and estrogen in their follicular fluid than intermediate or immature oocytes, while follicular fluid concentration of FSH, LH and PRL were significantly higher in the immature oocytes.

Luteal phase defect patients gave the lowest maturation rate and lowest fertilization rate.

Table(1):-The mean concentration of follicle stimulating hormone (mIu / ml) In the follicular fluid of different oocyte grades.

Mature oocyte	Intermediate oocyte	Immature oocyte
6.42±0.79	8.86±0.82	9.38±0.89

-Significantly higher in immature oocyte than mature oocyte $P<0.05$.
-No significant differ between immature and intermediate oocyte.

Table (2): - The mean concentration of Luteinizing hormone (mIu/ml) in theFollicular fluid of different oocyte grade.

Mature oocyte	Intermediate oocyte	Immature oocyte
2.195±0.57	3.50±1.02	4.70±1.05

-Significantly higher in the immature oocyte than mature oocyte $P<0.05$.
-No significant differ between immature and intermediate oocyte.

Table (3): - The mean concentration of Prolactin hormone (ng/ml) in theFollicular fluid of different oocyte grades.

Mature oocyte	Intermediate oocyte	Immature oocyte
7.145±1.345	10.8±1.26	13.46±1.47

-Significantly higher in immature oocyte than mature oocyte P<0.05.
-N0 significant differs between immature and intermediate oocyte.

Table (4): - The mean concentration of estradiol hormone (pg/ml) in theFollicular fluid of different oocyte grades.

Mature oocyte	Intermediate oocyte	Immature oocyte
2491.17±43	2201.15±53.5	2159.35±91.1

-Significantly higher in mature oocyte than intermediate or immature oocyte P<0.05.

Table (5): - The mean concentration of progesterone hormone (ng/ml) in the Follicular fluid of different oocyte grade.

Mature oocyte	Intermediate oocyte	Immature oocyte
61.12±2.76	50.63±1.80	48.13 ± 1.39

-Significantly higher in mature oocyte than intermediate or immature oocyte P<0.05

Table (6): - The results of in vitro fertilization of mature, intermediate andImmature human oocyte.

Oocyte grade	Number recovered	Number fertilized	Number unfertilized
Mature oocyte	84	73 (87%)	11 (13%)
Intermediate	45	29 (64.4%)	16 (35.5%)
Immature oocyte	45	13 (28.9%)	32 (71.1%)

-The percentage of fertilization is significantly higher in mature oocyte than
intermediate or immature oocyte $P<0.05$.
-The percentage of fertilization is significantly higher in intermediate than immature oocyte $P<0.05$.

Table (7): - The percent of oocyte maturation in luteal phase defect and control Group.

Group	No. of oocyte recovered	N0. of mature Oocyte recovered	No. of intermediate Oocyte	No. of immature Oocyte
Control group	95	60 63%	20 21%	15 15%
Luteal phase defect group	79	24 30.4%	25 31.6%	30 38%
Total	174	84	45	45

-Significantly higher maturation rate in the control group more than luteal phaseDefect group $P<0.05$.

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