Original Article

Pregnancy Outcome in the Luteal Defective Infertile Women Following ICSI, Embryo Transfer and Luteal Support Therapy.

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نتائج الحمل في النساء المصابات بقصور في وظيفة الجسم الاصفر بعد اجراء عمليات الاخصاب المجهري الاجباري (الاكزي) وزرع الاجنة وتثبيت الحمل.

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معهد الاخصاب الخارجي وابحاث الاجنة وعلاج العقم – جامعة بغداد مستشفى بغداد التعليمي كلية الطب بغداد-العراق .

ألملخص

عند حدوث خلل في تركيب ووظيفة الجسم الاصفر يؤدي الى قلة في افراز هرمون البروجسترون وعدم تهيئة الغشاء المبطن للرحم لزرع الجنين.

النساء المصابات بقصور في وظيفة الجسم الاصفر تشكل نسبة ٥٠% من النساء اللواتي لاتاتي لهن الحيض وبنسبة ٦٠% في النساء اللواتي يعانن من الاجهاض المتكرر.

ان هدف هذا البحث هو دراسة نتائج عمليات الاخصاب الخارجي المجهري الاجباري في النساء اللواتي يعانن من قصور بسيط او شديد في وظيفة الجسم الاصفر بعد اجراء عمليات زرع الاجنة.

قسمت النساء الى مجموعتين الاولى وتشمل هذه المجموعه المصابات بقصور بسيط والمجموعه الثانيه المصابات بقصور شديد في وظيفة الجسم الاصفر. تم اجراء عمليات تنشيط المبايض واستخراج البويضات والاخصاب المجهري الاجباري وزرع الاجنه في الرحم وتثبيت الحمل في هذه الدراسه وكذلك تم قياس تركيز هرمون البروجسترون والاستراديول والتستوستيرون وهرمون الاباضه(LH) والهرمون المحفز للحويصلات المبيضيه (FSH).

بالاضافه الى هرمون البرولكتين في السائل الحويصلي المبيضي(FOLLICULAR FLUID) للمجموعتين الاولى والثانيه. تم اجراء التحليل الاحصائي (ANALYSIS OF VARINACE) للنتائج واستخدمت الدلاله الاحصائيه المعنويه عندما كانت ($P<0.05$) .

بينت النتائج ان تركيز هرمون الاستراديول والبروجسترون كانت عاليه وبدلالة احصائيه معنويه في المجموعه الاولى مقارنة مع المجموعه الثانيه . كانت تراكيز هرمون البرولكتين والتستوستيرون عاليه وبدلاله احصائيه معنويه عاليه في المجموعه الثانية مقارنة مع المجموعه الاولى بينما كانت تراكيز هرمون (FSH)/(LH) متشابه وبدون دلاله احصائيه في المجموعتين الاولى والثانيه.

كان معدل الاخصاب المجهري الاجباري (الاكزي) بنسبة ٧٤,٤%في المجموعه الاولى و ٧٠,٠٧% في المجموعه الثانيه بينما كان معدل زرع الاجنه في المجموعه الاولى اعلى وبدلاله احصائيه معنويه مقارنة بالمجموعه الثانيه (٨٥,٠٢ % مقابل ٥٠ % على التوالي،$P<0.001$).

ان معدل الحمل لكل عملية زرع الجنين كان بنسبة ٣٠% في المجموعه الثانيه مقابل ٣٨% في المجموعه الاولى بينما كان معدل الغاء اجراء عمليات الاكزي بنسبة ١٣% بالمجموعه الثانيه مقابل ٤% في المجموعه الاولى .

استنتجت من هذه الدراسه بان استخدام عمليات الاخصاب المجهري الاجباري وتثبيت الحمل يؤدي الى تحسين معدلات الحمل وزرع الاجنه في النساء المصابات بقصور في وظيفة الجسم الاصفر وفي الرجال المصابين بضعف في القدره الاخصابيه للحيامن .

الكلما ت الدالة: (النساء المصابات في وظيفة الجسم الاصفر ، اسناد وظيفة الجسم الاصفر، ونتائج الاخصاب المجهري الاجباري).

Abstract:
Background: Defects in the formation and function of the corpus luteum result in a reduction of progesterone secretion and inadequate secretory transformation of the endometerium. Luteal defective cycles are recognized in 50% of unovulatory patients and 60% of women with recurrent abortions.
Objective: The goal of the present study was to study the outcome of ICSI in infertile women with mild and marked luteal defective cycles following embryo transfer.
Patients and Methods: Women with mild and marked luteal defective cycles were undergone ovulation induction, oocyte retrieval, ICSI and embryo transfer program. Follicular fluid progesterone, estradiol, testosterone were measured. The pregnancy rates in both luteal defective cycles (LDC) were recorded.
Results: The concentration of estradiol and progesterone were significantly higher in the mild LDC compared to marked LDC. The concentration of prolactine and testosterone were significantly higher in the marked LDC than mild LDC. The concentrations of FSH and LH were not significantly different between both groups of LDC. The ICSI rate was 74.4% in the mild and 70.7% in the marked LDC. The embryo transfer rate was significantly higher in the mild LDC compared to the marked LDC group (85.2% versus 50% respectively, $P<0.001$). The pregnancy rate per embryo transfer was 30.3% in the marked LDC compared to 38% in the mild LDC group. The patient cancellation rates were 13% and 4% in the marked and mild LDC groups.
Conclusions: The application of ICSI and luteal support therapy improves embryo implantation and pregnancy rates in infertile women with mild and marked luteal defective cycles and in men with severe teratozoospermia and poor sperm penetration score.
Key words: Luteal defective cycles, luteal support therapy, ICSI outcome.

Introduction:

The secretory activity of the corpus luteum and its functional life span are dependent on appropriate LH support (1). Interruption of LH pulsability by means of GnRH antagonist administration during various stages of the luteal phase induces rapid reduction of progesterone, estradiol and inhibin levels, followed by luteolysis and the onset of menses (2). Levels of FSH are suppressed during the luteal phase to reach the lowest levels during the entire cycle. FSH is not required for the maintenance of the corpus luteum. The combination of inhibin with estrogen and progesterone synergistically suppress FSH secretion and thus prevents the initiation of folliculogenesis during the luteal phase of the cycle (3).

The pathophysiology of the luteal defective cycles (LDC) has been postulated to encompass several different mechanisms, including; 1) abnormal follicular development leading to ovulation failure, luteinized unrupture follicle syndrom; 2) inadequate luteinization and subsequent deficient progesterone secretion; 3) hypocholesterolemia causing reduced progesterone secretion as a result of diminished supplies of steroid precursors; and 4) uterine abnormalities disrupting endometrial function, including submucosa myomas that may compromise endometrial vascularization, endometritis, and defects in steroid hormone receptors (4, 5).

Pharmacological manipulation of ovarian function by induction of ovulation with GnRH, menopausal gonadotropin, and clomiphene citrate is associated with luteal phase defect, ranging from 25 to 50 percent of cycles, has been claimed in women treated with clomiphene citrate. This abnormal endometrial maturation is postulated to be a major factor causing the low conception to ovulation rate and the increased spontaneous abortion rate associated with clomiphene therapy. Suboptimal FSH secretion or an inadequate LH surge in clomiphene-treated women can result in a corpus luteum with deficient secretory function (6). During in vitro fertilization and embryo transfer following controlled ovarian hyperstimulation results in excessive follicular growth and high serum concentration of progesterone and estradiol. This causes temporary luteal defective cycle in these women under going IVF and embryo transfer and suppress endogenous progesterone secretion, which may affect blastocyst implantation and pregnancy (7).

Luteal phase defective cycles (LPDC) are diagnosed in twenty five percent of infertile women, sixty percent of women with recurrent abortion and fifty percent of anovulatory females. Patients with LPDC are characterized by abnormal corpus luteum function associated with inadequate progesterone secretion (8-10). The application of luteal support therapy in the form of supplementary progesterone and/or human chorionic gonadotropin (HCG) have beneficial effect on embryo transfer, implantation, and maintenance of pregnancy in the stimulated IVF cycles in infertile women with LPDC (11). The objective of the present work was to study pregnancy rates in mild and marked luteal defective cycles in infertile women undergoing intracytoplasmic sperm injection (ICSI) and luteal support therapy using teratospermic semen with poor sperm penetration score.

Materials and Methods:

Patients:

The patients were divided into mild luteal defective cycle (LDC) and marked LDC. The number of the patients in the mild LDC group was 52 and in the marked LDC group were 46. Progesterone concentration was assayed on cycle day 21 and those women showing a progesterone concentration of less than 10 ng/ml and less than 3.0 ng/ml were considered to have mild and marked LDC respectively. Sperm penetration assay (SPA) using zona free hamster oocytes were used to examine male fertility. Men with poor SPA score were admitted to ICSI program in the present study and those with positive SPA score were admitted either to intrauterine insemination program or in vitro fertilization program in another study (12).

Semen Analysis and Sperm Penetration Assay (SPA): Semen analysis was performed to all male patients involved in the present study. Sperm concentration, motility, normal morphology and viability using vital stain were recorded. Sperm grade activity and motility index were calculated. Semen samples showing leukocytes and phagocytes were treated with appropriate antibiotic following semen culturing and sensitivity test for at least four weeks to control seminal fluid infection prior to ICSI operation. Superovulation was carried out in golden

hamsters with 20 mIU unit human menopausal gonadotropin on day one and two of the estrus cycle followed by 30 mIU of human chorionic gonadotropin (HCG) on cycle day four. Microsurgical recovery of the oocytes was performed after 16-17 hours following HCG treatment. The zona pellucida was removed chemically by trypsine enzyme. Normal mature hamster oocytes were inseminated in vitro culture system by patient sperm and sperm penetration and decondensation rates were recorded as good or poor scores for each male patient. Poor SPA score were admitted to ICSI program in the present study and those with good SPA score were admitted either to intrauterine insemination program or in vitro fertilization program in another study (13).

Ovulation Induction and ICSI Treatment: Ovulation was induced by human menopausal gonadotropin and human chorionic gonadotropin (HCG). The concentrations of FSH, LH, estradiol, progesterone and testosterone were assayed in the follicular fluid at the time of oocyte retrievals. Hypo-osmotic swelling test (HOST) was performed and those spermatozoa showed positive HOST was used for ICSI. Normal viable mature oocytes were used for sperm injection.

After ICSI and 24 hours before embryo transfer, the patients received progesterone therapy (400 mg/day by vaginal route cyclogest 400 mg, Hoechst-Roussal, Uxibridges, UK) plus 100 mg oral aspirin and the aspirin was given for 5 days after embryo transfer to support embryo implantation and to maintain pregnancy. The progesterone and aspirin treatments were continued for 12 weeks when positive embryo implantation was recorded following embryo transfer. The B-HCG was assayed on cycle day 14 following embryo transfer and those patients with positive B-HCG test (>12 mIU/ml) was considered to be pregnant. The assay was repeated after two days and if the second B-HCG value increased two-fold, transvaginal ultrasound was carried out 21 days later to see the fetal heart beat. The details of the procedure were described elsewhere (14).

ANOVA was used for the statistical analysis of the data. Chi-square and t-test were used to indicate the level of statistical significance when value was less than 0.05.

Results:

The concentrations of estradiol and progesterone in the follicular fluid were significantly higher in the mild LDC compared to the marked LDC group ($P<0.01$). The concentrations of prolactine and testosterone in the follicular fluid were significantly higher in the marked LDC compared to the mild LDC group ($P<0.05$). The concentration of the FSH in the marked LDC was significantly higher than mild LDC group (Table 1).

TABLE 1. Concentrations of reproductive hormone in the follicular fluid of mild and marked LDC infertile patients following ovulation induction and oocyte retrievals.

LDC Groups	LH IU/l	FSH IU/l	PRL ng/ml	Estradiol ng/ml	Progesterone ng/ml	Testosterone ng/ml	P-value
Mild	**0.12+/-0.02**	**4.2+/-1.4**	**21.1+/-8.6**	***512+/-84.5**	***13486.7**	**3.5+/-1.4**	***P<0.01**
Marked	**0.16+/-0.01**	***6.4+/-1.3**	****27.9+/-9.3**	**340+/-78.2**	**25870.2**	****6.7+/-2.5**	****P<0.05**

LDC=Luteal defective cycle. Data are mean +/- SEM. Asterisks show significant differences (*) P< 0.01 and (**) P < 0.5.

The results of ICSI outcome are shown in Table 2. The total number of the retrieved eggs was 336 and 280 in the mild and marked LDC groups respectively. The number of egg per patient was 6.5 and 6.3 in the mild and marked LDC groups respectively (P>0.05). The ICSI rate was 74.4% in the mild LDC group and 70.7% in the marked LDC group. The number of embryos transferred per patient was 4.3 and 3.8 in the mild and marked LDC groups respectively (P>0.05). The embryo transfer rate was significantly higher in the mild LDC group compared to marked LDC group (85.2% versus 51%, P<0.01). The pregnancy rate in the mild and marked LDC groups was 38% and 30.3% respectively (P.0.05, Table 2). The patient cancellation rate was 13% in the marked LDC group and 4% in the mild LDC group (P<0.05).

TABLE 2. ICSI and pregnancy outcomes in the mild and marked luteal defective cycle infertile patients.

LDC Groups	Eggs No.	No. egg per patient	ICSI percentage	ET per patient	ET rate	Pregnancy per ET	Cancellation percentage	P-value
Mild	**336**	**6.5(336/52)**	**74.4(250/336)**	**4.3(213/50)**	***85.2%**	**38%(81/213)**	**4%**	***P<0.01**
Marked	**280**	**6.3(280/46)**	**70.7(198/280)**	**3.8(152/40)**	**51%**	**30.3%(46/152)**	****13%**	****P<0.05**

LCD=Luteal defective cycle, ICSI=Intracytoplasmic sperm injection, ET=Embryo transfer.

Discussion:

Semen with poor sperm penetration score (SPS) was used for ICSI operation while those semen with good SPS was used either for IVF or intrauterine insemination depending on the patency of the Fallopian tubes. The majority of the semen in the present study were either severe teratospermic in nature but also some cases were unexplained infertility and used for ICSI treatment. All the infertile women had either mild or marked defective luteal cycles depending on the concentration of progesterone. The reason to assay reproductive hormone concentrations in the follicular fluid was to examine if these hormones have abnormal concentrations and may be play a role in the incidences of severity of luteal defective cycle (LDC). Intracytoplamic sperm injection (ICSI) is indicated after failure of other therapeutic methods including in vitro fertilization (IVF).

ICSI is a powerful tool for treatment of sperm dysfunctions. In patients with mild or severe teratozoospermia, ICSI was found to be superior to IVF technique (15). Recent evidence indicates that the outcome of ICSI is superior for implantation rate compared with IVF using a high-insemination concentration in cases with moderate and severe teratozoospermia, and with an adequate motile sperm fraction. It is possible that in IVF treatment cycle toxic reactive oxygen species may be released by abnormal sperm causing damage to the early embryos with good morphological score compared to ICSI (16). The overall after the transfer of embryos following ICSI seem to be similar to those of IVF. The presence of severe sperm abnormality and technical damage to the oocytes in ICSI cycle may compromise embryo development potential. It has been reported that ICSI has a higher fertilization rate compared to IVF and results with good embryos with better morphology (17).

The significant increase in the concentration of the FSH in the marked LDC group compared to mild LDC group may be due to reduction in the potency of the negative feed back mechanism which results in defective follicular development. The prolactine level was high ($P<0.05$) in the marked LDC women and this may have adverse effect on the maturation of the ovarian oocytes since high prolactine in the blood results in the reduction of gonadotropin concentrations and abnormality in the menstrual cycles and infertility. Testosterone concentration in the follicular fluid was also significantly higher in the marked LDC group versus mild LDC group. This hyper-androgenemia may be responsible for the induction of poor quality oocytes and also it may indicate insufficient function of aromatase enzymes that convert androgen to estradiol thus results in reduction in the estradiol concentration especially in the marked luteal defective cycle women (18-21). The ICSI rate although it was higher in the mild LDC group compared to marked LDC but the difference between both groups was not statistically significant and this may be an indication that ICSI is an effective tool for patients with poor sperm penetration assay (22). The embryo transfer rate was significantly higher in the mild LDC group versus marked LDC group and this may be due the effect of the unbalanced reproductive hormone levels in the follicular fluid which result in the production of poor quality oocytes in the marked LDC group and this may be also responsible for the higher cancellation rate in the marked LDC group (10). High concentration of estradiol in the mild LDC group is an indication of a good oocyte maturity and quality. Although the pregnancy rate per embryo transfer was relatively higher in the mild LDC group compared to marked LDC group but the differences were not statistically significant. These findings may be explained that either the sample size is small and need to be increased in the future experiment since chi-square analysis requires large sample size to reach a level of significance at $P<0.05$ or it is an indication that the luteal support therapy induces an adequate secretory transformation of the endometrium to support embryo implantation compromising the effect of luteal phase defect which is resulted from ovulation induction and suppression of ovarian function. In conclusion, the application of ICSI and luteal phase therapy improves pregnancy

rates in the mild and marked luteal defective cycle in infertile women and infertile men with marked terato-zoospermia and poor sperm penetration assay (14, 23-24).

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