

PREGNANCY RATE AFTER OVULATION INDUCTION, SPERM INTRAUTERINE TRANSFER (SIUT) AND LUTEAL SUPPORT THERAPY IN LUTEAL PHASE DEFECT (LPD) INFERTILE PATIENTS.

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Summary:

Background: It is well known that the early removal of corpus luteum (CL) in pregnant women results in abortion . Defects in the function of CL lead to deficiency in the secretion of progesterone which adversely affect human embryo implantation .

Aim of the work: 1)to determine progesterone concentration in the luteal phase defect (LPD) patients complaining from infertility and 2) to evaluate the clinical value of ovulation induction, sperm intrauterine insemination SIUT and luteal support therapy in the treatment of LPD patients.

Patients & Methods: One hundred and twelve LPD patients were involved in this study. The progesterone concentrations were performed by radioimmunoassay method on cycle day 21. Patients were considered to have severe LPD when progesterone concentration was 3.56 ng/ml and mild LPD when the progesterone concentration was less than 8.63 ng/ml. Those patients who had progesterone concentration of more than 10 ng/ml were considered normal (without LPD).

Ovulation induction was induced by clomiphene citrate (100mg/day for five days) and human menopausal gonadotropin (300 international units for another five days) and human chorionic gonadotropin (HCG) treatments. Standard technique for in vitro activation of human sperm and sperm intrauterine insemination (SIUI) were performed. Following IUI the patients were received 1500 IU of HCG on cycle day 14,17,20 and 23.

Results: The pregnancy rate in the severe LPD group was significantly lower ($P < 0.05$) than that of control and mild LPD groups. The pregnancy rate in the control (without LPD) and mild LPD group was significantly not different ($P > 0.05$). This indicates that the outcome of luteal support therapy following ovulation induction and SIUT was significantly improved when compared to control group .

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Introduction

It is well known that the early removal of corpus luteum (CL) in pregnant women results in abortion (1). This is because embryo implantation in human uterine endometrium is dependent on adequate secretion of progesterone (P) hormone from CL (2). Therefore defects in the function of CL lead to deficiency in the secretion of progesterone which adversely affect human embryo implantation (3,4).

Luteal phase defect (LPD) is characterized by deficient in the estradiogenesis primarily of progesterone during luteal phase of the cycle (5). Bradford and Donna, (6) reported that the incidence of LPD was 63% in patients complaining from infertility. The causes of LPD are poor follicular

development, insufficiency of CL and failure of the uterine lining to respond to normal level of progesterone (7). The normal luteal phase length is defined by the interval between the LH peak and onset of menses and ranged from 11 to 15 days. The short luteal phase is defined as a luteal phase length of ten days or less (8). The inadequate luteal phase is associated with normal length but with abnormal low progesterone concentration throughout the luteal phase. A level less than 10 ng/ml indicates the progesterone production in the luteal phase is inadequate (9).

The objectives of the present work were 1) to determine progesterone concentration in the LPD patients;2) to evaluate the clinical value of ovulation induction, sperm intrauterine insemination (SIUT) and luteal support therapy in the treatment of LPD infertile patients.

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PATIENTS:

One hundred and twelve (112) infertile female patients complaining from LPD as a cause of female infertility with sixty-four (64) infertile females without LPD problem were included in this study at Saddam Institute for Embryo Research and Infertility Treatment, Department of In Vitro Fertilization. In order to assess the function of corpus luteal (C.L) in these patients, serum progesterone (P) was measured on day 21 of the previous cycle. Hormonal analysis of FSH, LH, Prolactin and estradiol was also measured. Radioimmunoassay technique was used according to standard technique.

Patients showing a progesterone concentration of less than 3.56 ng/ml on cycle day 21 was considered to have severe LPD (severe LPD group), and patients showed progesterone concentration of less than 8.63ng/ml was considered to have mild LPD (mild LPD group). Those infertile patients with normal progesterone concentration was considered normal without LPD (control group) and had progesterone concentration of more than 12ng/ml. The husbands of these patients had normal sperm function tests with normal reproductive hormones profile and normal thyroid hormones function. These husbands had also normal sperm penetration assay rate and they were fertile. The mean age of females patients in this study was 33.5 ± 3.40 years with a range of 25-40 years. The mean duration of infertility was 8 ± 1.86 years with a range of 3-10 years.

OVARIAN STIMULATION:

Ovarian stimulation was induced by clomiphene citrate (clomid) 50mg twice daily started from cycle day 2 to 6 followed by 300 international units (IU) of human menopausal gonadotropin (Humagon: hMG) daily from cycle day 7 to 11. The dose of the hMG was dependent on the response of the ovarian follicular development to the treatment protocol. The growth of the ovarian follicles was measured by ultrasonography and the determination of blood estradiole concentration was performed by radioimmunoassay technique. When the follicular diameter was more than 18mm and the concentration of estradiole was 200 to 250 Pg/ml/follicle, a dose of 10000 IU of human chorionic gonadotropin (hCG) was administered, intramuscularly to infertile patients, (Figure 1).

SPERM ACTIVATION:

Ejaculated semen was allowed to liquefy at 37°C in incubator. Baghdad culture medium (BCM) with 9% inactivated wife serum at Ph 7.4 and 285 to 290 mosmol under 5% CO₂ in air at 37°C was used for sperm activation. One ml of the ejaculate

after liquefaction was mixed with 1.5ml of the BCM. The mixture was centrifuged for 10 minutes at 400g. The supernatant was removed and the sperm pellet was resuspended in one ml fresh BCM for a period of 30 to 60 minutes in the CO₂ incubator after the end of culture time. The concentration of the sperm cells, the motility, sperm grade activity motility index and normal sperm morphology were recorded under high power Olympus microscope. The technique of sperm activation was carried out under sterile conditions to avoid any possible contamination (5).

SPERM INTRAUTERINE TRANSFER (SIUT):

A 100 microliter of the supernatant fluid was examined for sperm concentration, motility, grade activity and normal sperm morphology. One to 1.5 ml of the supernatant culture was aspirated containing sperm concentration of $\geq 20 \times 10^6$ sperm/ml by a Fradymen catheter under sterile conditions. The external cervical os was cleaned with fresh culture medium. The external cervical os was exposed by speculum. The catheter was introduced into the uterine cavity via the cervical canal and special care was taken to avoid any bleeding in the cervical canal wall during the passage of the catheter. The SIUT was performed 24 to 30 hr after HCG injection.

The sperm cells were transferred to the uterus near the tubo-uterine junction. Following SIUT with hyper-active motile sperm, the patient remained in the lithotomy position for a period of 20 minutes before discharge. The patients were received 20mg hyoscine N-butyl bromide B.P. about 10 minutes before SIUT and 1500 IU HCG on cycle day 14, 17, 20 and 23. The B-HCG and progesterone hormones were assayed after 14 days of SIUT. The appearance of gestation sac with viable heart beat after 7 weeks of last menstrual period was considered to established clinical pregnancy.

RESULTS:

The program of ovulation induction and SIUT followed by Luteal support therapy is shown in figure 1. The sperm function test of the husband of the LPD infertile patients is shown in table one. The sperm concentration ($46.32 \pm 12.76 \times 10^6$ /ml), sperm motility (60%), sperm grade activity (3.80 ± 1.04), sperm motility index (2.28 ± 0.26), sperm normal morphology (64%), sperm viability (75%) and Leukocytes and phagocytes concentration (1.03×10^6 /ml ± 0.15) were within the normal range of seminogram.

The progesterone concentration in the serum of the LPD patients was significantly ($P < 0.001$) lower than that of control group. The mild LPD group had progesterone concentration on cycle day 21 higher value compared to severe LPD group (8.63 versus 3.56, $P < 0.01$, Fig.2). When both values

of progesterone in mild and severe LPD groups combined with each other showed significantly lower values than that of control group (6.09 Versus 16.74, $P<0.001$ respectively, Figure 3). Table number two shows pregnancy rates in LPD patients following ovulation induction, intrauterine insemination and Luteal support therapy. The pregnancy rate in the severe LPD group was significantly lower than that of control and mild LPD groups ($P<0.05$). The pregnancy rate between the control and mild LPD groups was non-significantly different from each others ($P>0.05$). When both values of mild and severe LPD-pregnant groups were compared to that of control, the values were significantly different (68.42 Versus 60, $P<0.05$, Figure 4). The progesterone concentration and B-HCG concentration were significantly higher in the pregnant group compared to non-pregnant group (37.86 ng/ml, 248.30 mIU/ml Versus 3.50 ng/ml and 2.16 mIU/ml, $P<0.001$, Table 3).

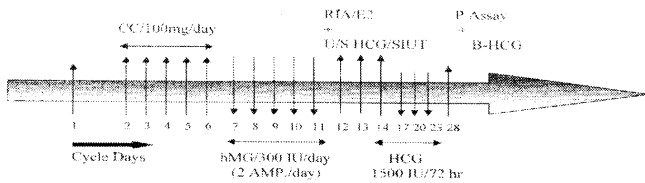


Fig.1. Ovulation induction in LPD patients by clomiphene citrate, human menopausal gonadotropine and human chorionic gonadotropine plus sperm intrauterine insemination

- E2: Estradiol
- P: Progesterone
- HCG: Human chorionic gonadotropine: (10000 IU to induce ovulation)
- U/S: Ultrasound: Vaginal probe
- CC: Clomiphene citrate: 50mg/tablet
- RIA: Radioimmunoassay
- hMG: Human menopausal gonadotropine:75 IU FSH+75 IU LH
- B-HCG:Beta-human chorionic gonadotropin
- SIUT: Sperm Intra-Uterine-Transfer

Table 1. Seminal fluid parameters of the males of LPD females.

Sperm Concentration	46.32 X 10 ⁶ /ml ± 12.76
Sperm Grade Activity	3.80 ± 1.04
Sperm Motility Percent	60%
Sperm Motility Index	3.28 ± 0.26
Sperm Normal Morphology	64%
Sperm Viability	75%
Leukocytes and P. agocytes	< 1.03 X 10 ⁶ /ml ± 0.15

Data are means±SEM

Total number of males: 176

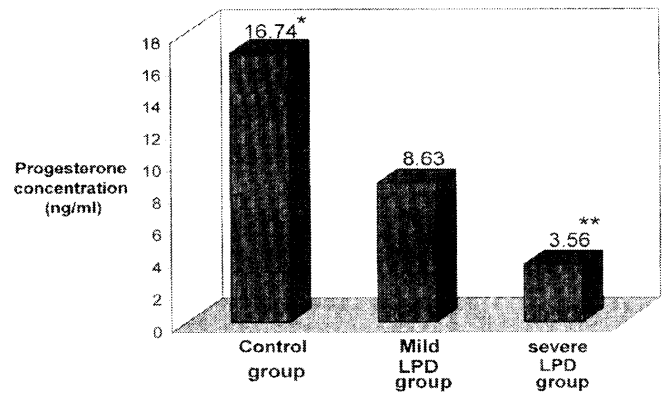


Figure 2. Progesterone concentration in the serum of LPD patients on cycle day 21 (control without LPD and mild and severe LPD groups).

* $P<0.001$ significantly different from other groups.

** $P<0.01$ significantly different from mild LPD group.

LPD: Luteal phase defect.

Number of patient per group=30

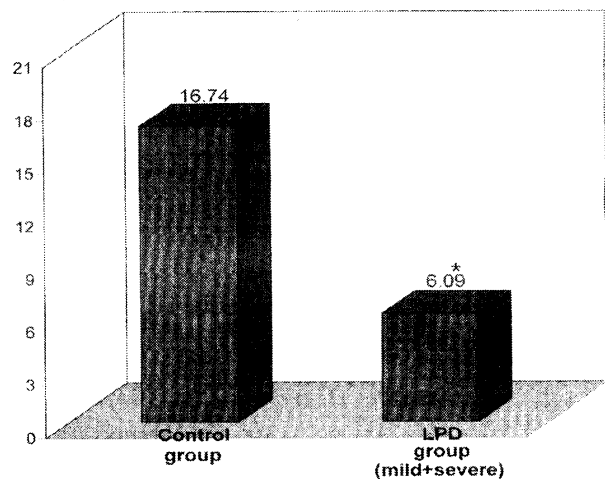


Figure 3 . Progesterone concentration in the blood of LPD groups both mild and severe and non-LPD group (control).

* $P<0.001$ Significantly different from control group.

No. of patients in LPD groups=60

No. of patients in control group=30

Table 2. Pregnancy rate per patient in LPD patients following ovulation induction, sperm intrauterine transfer and luteal support therapy.

Groups	Control W/O LPD	Mild LPD	Severe LPD
Patients Number	38	36	34
Pregnant Patients	26	24	18
Pregnancy Rate	68.42%*	66.66%	52.94%**

* P< 0.01 Significantly different from severe LPD group.

**P< 0.05 Significantly different from mild LPD group.

LPD=Luteal phase defect.

Total number of infertile patients:176

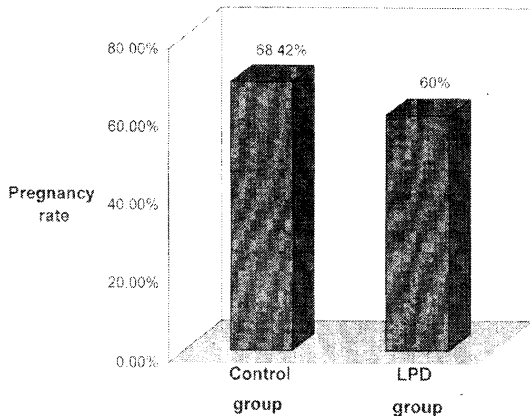


Figure 4. Pregnancy rate in the control and LPD group following ovulation induction, sperm intrauterine transfer (SIUT) and luteal support therapy.

Number of patients per control group = 30

Number of patients per LPD group = 42

P<0.05 : Significantly different from control group.

Table 3. Progesterone and B-HCG concentration in the serum of luteal support pregnant and non-pregnant groups following 14 days of sperm intrauterine transfer.

Hormones	Pregnant	Non-Pregnant
Progesterone (ng/ml)	37.86*±3.64	3.50±1.31
B-HCG (mIU/ml)	248.30*±14.72	2.16±1.80

*P< 0.001 Significantly different from non-pregnant group.

Number of patient per group = 24

DISCUSSION:

Luteal phase progesterone (P4) supplementation has been shown to have clinical value in the treatment of deficient P4 production. These treatments have been documented by repeated endometrial biopsies performed after intramuscular (I.M) or vaginal P4 supplementation (10). This supplemented P4 has a direct effect on uterine endometrium and the endometrial architecture has shown to be appropriate in phase development and pregnancy and miscarriages rates have seemed to be normal (11). The I.M P4 supplementation causes a delay of menses in most women (12).

The objective of the present research was to apply human chorionic gonadotropin (HCG) treatment in LPD patients to induce P4 production from corpus luteum (CL). This indirect stimulatory action of HCG on CL is more physiologic in nature. This was followed by I.M P4 supplementation in oil after the last HCG injection to support embryo implantation, (13,14). The concentrations of P4 in the serum of mild and severe LPD infertile patients and control group were similar to those reported by other investigators (5,7,9).

The pregnancy rate in the severe LPD group following ovulation induction, intrauterine transfer (SIUT) and luteal support therapy was significantly lower compared to that of mild LPD and control groups. This may indicate that the dysfunction in the CL was marked as evidenced by very low P4 concentration on cycle day 21 in the severe LPD group. Similar results were reported by other workers (5,15). In this group the prolactin concentration was high and this may have inhibitory action on LH hormone surge which result in poor follicular growth and unadequate CL development that affect negatively its secretory capacity (16,17).

There was no significant difference in the pregnancy rate between control and mild LPD groups. This may be due to the efficiency of the luteal support therapy to maintain sufficient P4 concentration in order to support embryo implantation and pregnancy (13,14). Progesterone administration may maximize the uterine tissue progesterone production and inhibit uterine contraction and support embryo implantation (18,19).

The high concentrations of progesterone and BHCG in the blood of pregnant group may be due to the combine stimulatory effects of luteal support therapy and embryo itself on anterior pituitary gland. This results in high LH secretions and subsequently lead to adequate CL function and sufficient progesterone secretion (5,19).

The application of ovulation induction, sperm intrauterine transfer (SIUT) and luteal support therapy treatment in the luteal phase defect in infertile patients (providing that the husband have normal sperm function and sperm penetration rate) resulted in significant improvement in pregnancy rates. These results were confirmed by other researchers (5,15,20).

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