Ministry of Higher Education and Scientific Research
Al-Nahrain University
High Institute for Infertility Diagnosis and Assisted Reproductive Technologies

Study the Effects of Intrauterine flushing with Low Dose Human Chorionic Gonadotrophin on Endometrial Receptivity and ICSI Outcome

OCTOBER 16, 2024

Chapter Three Patients, Materials and Methods

A prospective comparative study was conducted in the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University in Baghdad, from January 2023 to January 2024. Ninety Iraqi women were included in this study. Every patient gave her written informed consent before taking part in the study figure (3-1), which was approved by the Ethical Committee of High Institute for Infertility Diagnosis and Assisted reproductive Technologies, Al-Nahrain University. They were evaluated twice during the study period and they did not receive any monetary compensation for their participation.

3.1. Patients

A study involved a total of ninety women who were selected from those attended the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies.

3.1.1. Patient classification

- Group A**:** patients who receive (700IU) of intrauterine hCG
- Group B: patients who receive (500IU) of intrauterine hCG
- Group C: Control group patients did not receive any treatment.

3.1.2. Inclusion criteria

• All couples undergoing IVF / ICSI protocols reaching the day of embryo transfer with grade 1 (G1) embryos.

• Age of women was (18-40) years old.

• Infertility due to female factors: tubal blockage, un ovulatory cycles, unexplained infertility.

• Infertility due to male factors.

• fresh cycle transfer

3.1.3. Exclusion criteria

• All cases of endometrioses whether suspected clinically or diagnosed laparoscopically.

• Cases of congenital anomalies of the reproductive system.

• Patients aged more than 40 year

• Those not having G1 embryos.

• Cases with chronic systemic diseases (diabetes, blood disease, hypertension, connective tissue and autoimmune diseases, asthma, class II and III obesity, thyroid disturbance).

3.2 Materials equipment and tools

3.2.1. Equipment

The current study utilizes the tools, instruments, chemical substances and kits, outlined in Tables (3-1), (3-2) and (3-3).

Table (3-1) Apparatus and Equipment

Apparatus & Equipment	Company	Country
Air Incubator	**Memmert**	**Germany**
Automatic Pipette	**Slammed**	**Germany**
Centrifuge	**Eppendorf**	**Germany**
CO2 Incubator	**Galaxy47/Eppendorf/**	**Germany**
Collecting Tubes 1.5 ml	**AFCO**	**Jordan**

Dry Incubator	**Fisher scientific**	**USA**
EZ- tip RI	**Cooper surgical fertility companies, Malov**	**Denmark**
EZ-grip RI	**Cooper surgical fertility companies, Malov**	**/Denmark**
Flexible catheter (embryo transfer catheter	**Gynetics**	**Ireland**
Freezer	**Concord**	**lebanon**
Gel &Clot Activator Tubes	**AFCO**	**Jordon**
Glass Pasteur Pipette(150mm)	**Poulten & Graf LTD**	**England**
ICSI manipulator	**Eppendorf/Nikon Te2000-4**	**Germany**
IUI catheter	**Gyntics**	**Beligum**
Laminar Flow Cabinet	**K-Systems**	**Denmark**
Light microscope	**MEIJI**	**Japan**
Medical suction machine	**ARDO**	**Switzerland**
Micropipette of 5-50, 100-1000µl	**Thermo Scientific Nunc IVF Product**	**Denmark**
Microplate reader capable of measuring absorbance at 450 nm	**Human reader**	**Germany**
Mini vidas Automated Immunoassay	**BIOMRIEUX/**	**Italy**
Ovum aspiration needle	**Cook®**	**Australia**
Pasteur pipette and tubes	**Eppendorf**	**Germany**
Pipette Tips (Yellow, Blue)	**Brand**	**USA**

Pipette Tips (Yellow)	**Brand**	**Germany**
Plain Tubes	**AFCO**	**Jordon**
Plastic rack	**AFCO**	**Jordon**
RI holding pipette	**Cooper surgical fertility companies, Malov**	**/ Denmark**
RI Spiked Injection Pipette	**Cooper surgical fertility companies,Malov**	**Denmark**
Semi-automatic microprocessor controlled photometer	**Humalyzer Primus**	**/Germany**
Scale (weight &height measure)	**Health scale**	**China**
Slides and cover slips		**Germany**
Stereomicroscope	**Olympus/SZx16**	**Japan**
Syringes (5ml, BD Discardit^{TM}II)	**Becton Dickinson**	**USA**
Test tubes, Falcon petridish, Small dish, Center well dish, Four well dish, ICSI dish	**Thermo Scientific Nunc IVF Product**	**Denmark**
Vaginal Sonography	**Vesana Balance**	**china**
Wash bottle	**Brand**	**India**

3.2.2. Chemicals and drugs

The chemicals and drugs that used in this study are summarized in Tables (3.3 - 3.4).

Table 3.2: Chemicals and their manufactures

Chemicals	Manufacturing company/Country
Ferticult TM Aspiration	FertiPro NV/Belgium
FerticultTM Flushing medium	FertiPro NV/Belgium
FerticultTM Mineral oil	FertiPro NV/Belgium
Gain medium/ Early	FertiPro NV/Belgium
Hyaluronidase medium 80IU/ml	FertiPro NV/Belgium
PVP 10% in Flushing medium	FertiPro NV/Belgium

Table (3-3): Drugs used in ICSI and their manufactures : Antagonist protocol

Trade Name	Chemical name	Company & country
Gonal f	Recombinant FSH 75u injection	Merk, Switzerland
Cetrotide®	Cetrorelix acetate for injection 0.25 mg	Merk, Switzerland
Oviltrelle	250 microgram hCG	Merk, Switzerland
Cyclogest	Natural Progesterone	CoxPharmaceuticals , Barnstaple, UK
hCG	Urinary human chorionic gonadotropin	Diclaire
Triptorelin	(Decapeptyl®) 0.1 mg vial	Ferring/ Germany

Table 3.4 Chemicals and kits

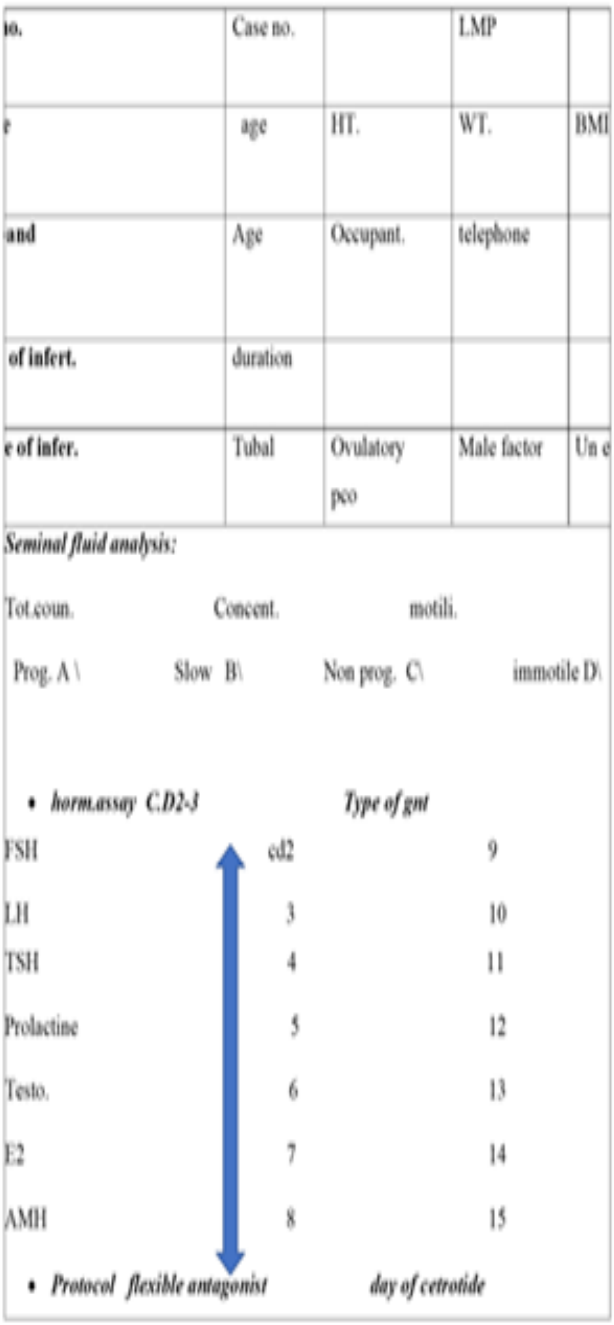
Chemicals and Kits	Company	Country
Human TNF Kit and IL_10 ELISA Kit	**R&D Systems biotechne`brand**	**USA**
Liaison FSH kit	**Liaison**	**Italy**
Liaison LH kit	**Liaison**	**Italy**
Liaison TSH kit	**Liaison**	**Italy**
Liaison PRL kit	**Liaison**	**Italy**
TOSOH E2 Kit	**TOSOH AIA 600II**	**Japan**

3.3. Methods

3.3.1. Patients' assessment and study design

A comprehensive medical, surgical, and obstetrical history was gathered from couples experiencing infertility, as per a structured questionnaire illustrated in figure (3-2). Thorough examinations were conducted on infertile women, encompassing both general and gynecological assessments. Height and weight measurements were taken to calculate the body mass index (BMI) using the formula BMI = weight (kg) / [height (m)]2. Initial hormonal evaluations were performed on either the second or third day of the menstrual cycle for all female subjects. Procedures such as transvaginal ultrasound, saline infusion sonography (SIS), and hysterosalpingography (HSG) were carried out to evaluate the uterine cavity, confirm tubal patency, and in some cases, laparoscopy was employed to assess tubal patency and exclude pelvic abnormalities. Seminal fluid analysis was conducted for the male partners. All infertile women enrolled underwent the application of the Gonadotropin Releasing Hormone (GnRH) antagonist ovarian hyperstimulation protocol, known as the flexible protocol. The follow-up of

patients included serial vaginal ultrasound and monitoring of serum estradiol levels. Subsequently, ovum pick up (OPU) was carried out post ovulation triggering approximately 35-36 hours later. Doppler ultrasound, along with measuring serum TNF and IL-10 levels, was performed for all participants on the day of OPU, followed by additional measurements on the day of embryo transfer. Evaluation of oocytes' morphology and recording of maturation rate (MR) were conducted, leading to Intracytoplasmic Sperm Injection (ICSI) of apparently normal Metaphase II (MII) oocytes. The morphological assessment of embryos was done, and the count of different embryo grades was noted. Luteal phase support, embryo transfer, and serum beta-human chorionic gonadotropin (B-hCG) evaluation were done on day 14 post embryo transfer, with the recording of biochemical pregnancy rate (Figure 3.3).



o.	Case no.		LMP	
e	age	HT.	WT.	BMI
and	Age	Occupant.	telephone	
of infert.	duration			
e of infer.	Tubal	Ovulatory pco	Male factor	Un e

Seminal fluid analysis:

Tot.coun. Concent. motili.

Prog. A \ Slow B\ Non prog. C\ immotile D\

- *horm.assay C.D2-3* *Type of gnt*

FSH	cd2	9
LH	3	10
TSH	4	11
Prolactine	5	12
Testo.	6	13
E2	7	14
AMH	8	15

- *Protocol flexible antagonist* *day of cetrotide*

- *Protocol flexible antagonist* *day of cetrotide*
- *type of trigger* *cd* *dose*

Ultrasound	*Base line US*	*Day of pick up*	*day Embryo transfer*
E.T. hypo echoic (triple)			
Hyper echoic (non-triple)			
Sub endometrial flow RI			
PI			
V MAX			
V MIN			
VS/VD			

Number of oocyte *M1* *M2* *GV* *abnormal*

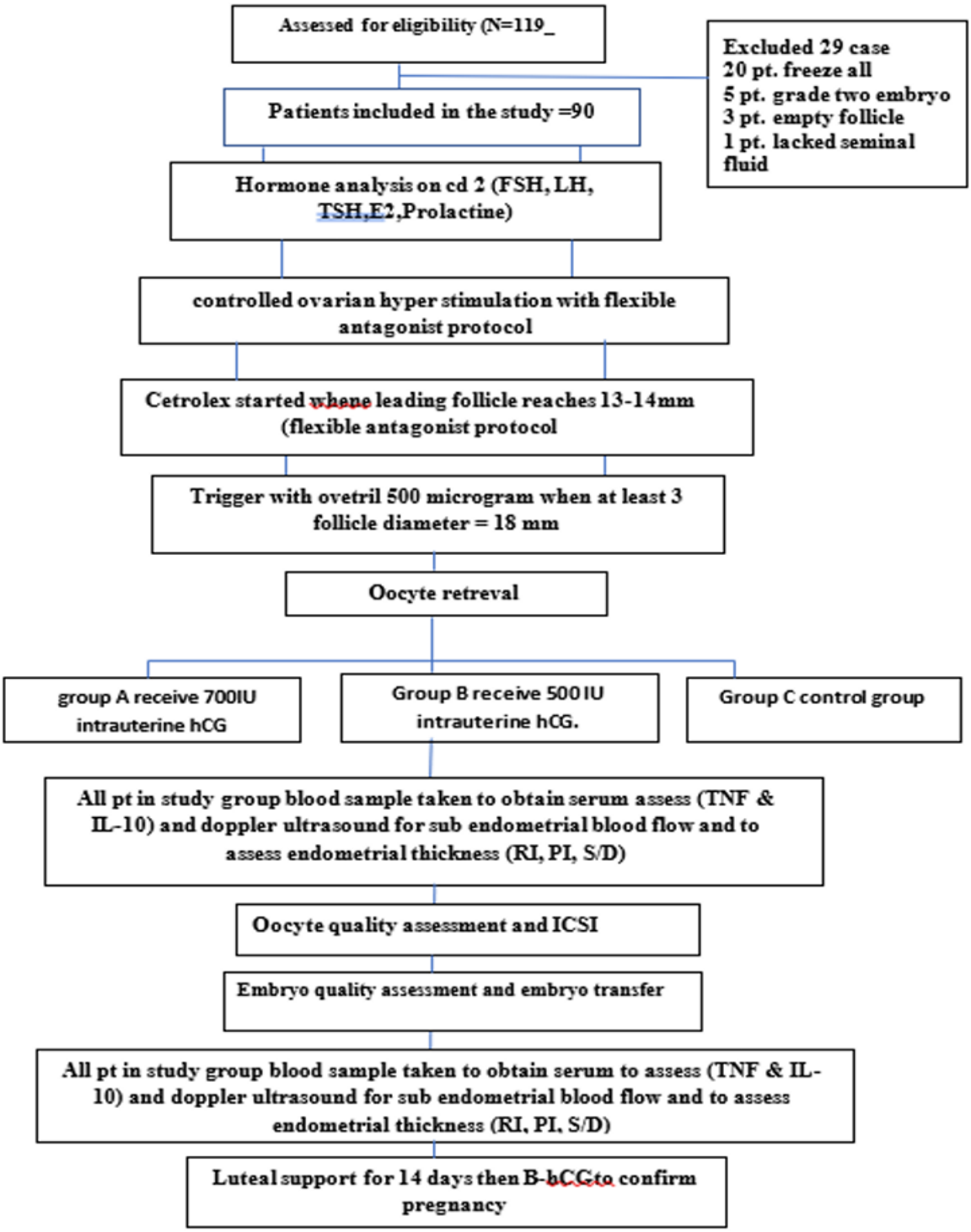
	Day 1	*2*	*3*	*4*	*5*
No. of embryo & grade					

Embryo. Transfer *no.* *day* *grade*

Biochemical marker in blood	*day of pick up*	*day of transfer*
T.N.F.		
Interleukine-10		

Pregn. test

figure (3-2): Questionnaire



Figure(3-3): study design

3.3.2. Anthropometric measurement

The anthropometric parameters examined in the research primarily encompassed Body Mass Index (BMI), as determined by the subsequent criteria.

The computation of BMI involves dividing the weight in kilograms by the square of the height in meters (kg/m2). Subsequently, in accordance with WHO standards, females are categorized based on their BMI as follows:

those with a BMI ≤18.5 are classified as underweight,

individuals with a BMI of 18.5-24.9 are considered to have a normal weight,

those falling within the 25-29.9 range are classified as overweight, and

those with a BMI ≥ 30 are categorized as obese. The formula for calculating BMI is expressed as BMI = Weight in Kg ÷ (height in m)2.

3.3.3. Basal fertility workup

- Clinical and gynecological examinations were conducted along with basal ultrasound scanning via transvaginal route in order to rule out any potential abnormalities.
- Baseline hormonal evaluation was carried out during the early follicular phase (day 2 or 3 of the menstrual cycle) encompassing Follicle-Stimulating Hormone (FSH), luteinizing hormone (LH), and estradiol (E2). The assessment of hormones was executed employing an enzyme-linked fluorescent assay (ELFA) method, utilizing Minivids analysis apparatus (BIOMRIEUX/France).
- Viral studies were conducted for both partners.

3.3.4. Intracytoplasmic sperm injection program

3.3.4.1.a. The flexible antagonist Protocol

A flexible antagonist protocol type was implemented for ICSI across all participants, with ovarian stimulation commencing once all parameters were within normal ranges. These parameters included an endometrium thickness of less than 5 mm, absence of any follicle exceeding 10 mm in the ovaries, and E2 levels below 50 pg/ml. The protocol entailed ovarian stimulation through daily subcutaneous injections of rFSH (Gonal F, Merk Serono)®, each containing 75IU per ampoule. The initial gonadotropin dosage was tailored based on individual factors such as age, antral follicle count, BMI, and previous responses to ovulation induction. Administration began on the second day of the menstrual cycle. Additionally, some participants were administered HMG vials (Menogon, Ferring Pharmaceutical) intramuscularly, each containing 75 IU of FSH and LH per vial, in accordance with their clinical needs. To address patient-specific requirements, a flexible regimen of GnRH antagonist (Cetrorelix acetate 0.25 mg, s/c Cetrotide®, Merck, Switzerland) was initiated once the primary follicle reached 13-14 mm. Serum E2 levels were also monitored throughout the procedure.

3.3.4.1.b.Triggering of ovulation

Induction of final oocyte maturation occurred when a minimum of three dominant follicles reached a size equal to or greater than 17–18 mm in diameter through the administration of subcutaneous recombinant hCG (Ovitrelle®, 6500 IU Merk serono, Switzerland), or initiation was facilitated by the use of a "dual trigger" comprising human chorionic gonadotropin (hCG) in combination with gonadotropin releasing hormone agonist (GnRH-a), specifically triptorelin (Decapeptyl; Ferring Pharmaceuticals) administered subcutaneously (. Le MT, et al. 2019)

3.3.4.2. Transvaginal ultrasound guided oocyte retrieval

A specialist recovered the oocytes 34–36 hours post-triggering, just prior to follicular rupture. Patients were admitted to the operating theatre in a fasting state, positioned lithotomy with an empty bladder, administered general anesthesia, and subjected to vaginal lavage with normal saline. Oocytes were retrieved from individuals under transvaginal ultrasound guidance using a delicate single lumen aspiration needle (Gynetics®, Belgium) connected to a Wallace oocyte recovery system. The opposite end of the needle was linked to a suction apparatus operating at 120-130 mm Hg. (Atzmon et al., 2020). Every follicle in both ovaries was aspirated and promptly transferred to the embryologist for meticulous examination to ascertain the constitution of the collected cumulus-oocyte complexes. Subsequent to completion, the needle was withdrawn, ensuring successful hemostasis. The entire procedure typically lasted between 20-30 minutes. Subsequently, patients received analgesics, antibiotics, and luteal phase support in the form of progesterone (Zheng et al., 2019).

3.3.4.3. Oocyte grading

The categorization of oocytes in terms of their maturity grade was conducted subsequent to the removal of the corona layers and cumulus. Subsequently, the oocytes underwent a thorough washing process and were examined under an inverted microscope to assess their stage of maturity and overall integrity. The evaluation of oocyte nuclear maturity relied on the presence of an extruded first polar body (PBI) within the perivitelline space and the absence of a germinal vesicle (GV). The germinal vesicle (GV) denotes the least mature oocyte where this circular structure is situated within the oocyte (refer to Figure 3.4, A). The MI stage represents an immature oocyte characterized by the absence of a polar body or germinal vesicle, serving as an intermediary phase between GV and MII

(Coughlan C, Ledger B, 2011) (refer to Figure 3.4, B). MII signifies the most mature oocyte containing a polar body (refer to Figure 3.4, C).

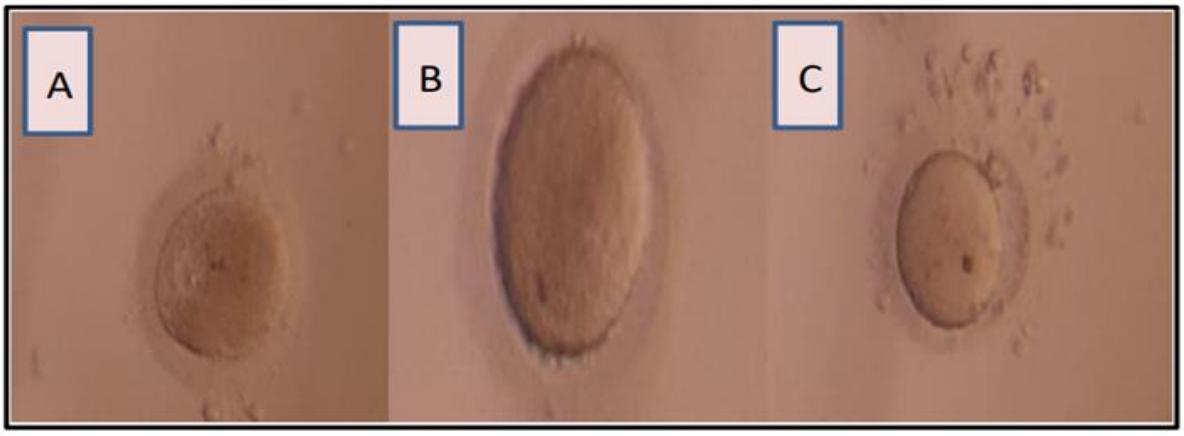


Figure 3.4 depicts oocytes exhibiting varying degrees of maturity; (A) illustrates an oocyte classified as Germinal vesicle grade of maturity; (B) showcases an oocyte at Metaphase I grade of maturity; and (C) displays a typically mature metaphase II oocyte. This information was sourced from the esteemed High Institute for Infertility Diagnosis and Assisted Reproductive Technologies at AlNahrain University in Baghdad, Iraq.

According to Ozgur et al. (2015), the maturity index, or the proportion of mature oocytes for each participant, was determined using the following formula: Maturity index = (Total number of mature oocytes (MII) / Total number of oocytes retrieved) × 100.

3.3.4.4. Fertilization and Embryo Culture

3.3.4.4.a. Sperms Preparation

Usually before OPU, semen sample was prepared for sperm extraction. The sample was collected in a private room near the laboratory in order to limit the exposure of the semen to fluctuations in temperature and to control the time between collection and analysis. The sample was collected after a minimum of 2 days and a maximum of 7 days of sexual abstinence. The sample was obtained by

masturbation and ejaculated into a clean, wide-mouthed container made of glass or plastic, from a batch that had been confirmed to be non-toxic for spermatozoa. The specimen container was kept at ambient temperature between 20 °C and 37 °C to avoid large changes in temperature that may affect the spermatozoa after they were ejaculated into it. It was labeled with the man's name, identification number, date and time of collection. The specimen container was placed in the incubator (37 °C) while the semen liquefied. In men with azoospermia Testicular Sperm Extraction (TESE) is a surgical sperm retrieval procedure used as infertility treatment. It was done by removing a small portion of tissue from the testicle under general anesthesia and extracting the few viable sperm cells presented in that tissue for ICSI.

3.3.4.4. b. Intracytoplasmic sperm injection procedure

At the IVF lab, aspirated follicles were immediately examined in a petri dish. The oocyte was retained in the CO2 incubator at 37°C for one to two hours after flushing. Following that, each oocyte was graded and stripped in the laminar flow cabinet.

The mature oocyte was grasped using a specialized holding pipette, and one chosen sperm with normal morphology was immobilized and picked up using an ICSI microscope and a very thin, pointed, hollow needle. The oocyte's shell was then gradually punctured with the needle. (with PB at 12 or 6 o'clock) into its cytoplasm (Figure 3.5). To reduce the possibility of chromosomal or spindle damage, the genetic material is kept away from the injection site because it is anticipated to be adjacent to the PBI. Once the sperm were carefully injected into the cytoplasm and the needle was taken out, the sample was kept in a CO2 incubator to await the potential result of fertilization.

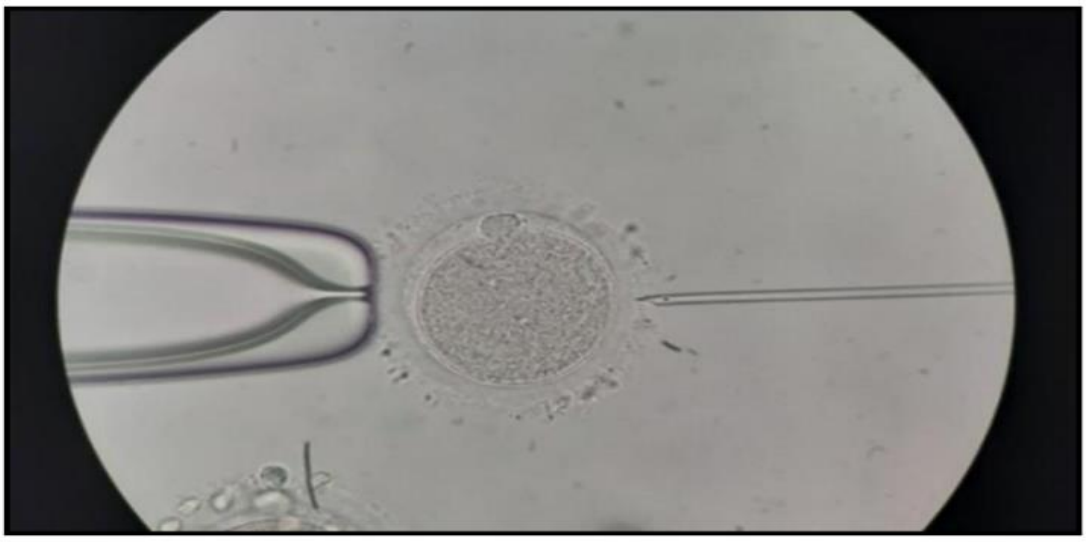


Figure 3.5: ICSI Micromanipulation, Polar body at 12 or 6 o'clock in ICSI images, adapted from Al Nahrain University, Baghdad, Iraq's High Institute for Infertility diagnosis and Assisted Reproductive Technologies

3.3.4.5. Evaluation of fertilization and embryo grading :

Figure 3.6 illustrates the assessment of injected oocytes, which was carried out 16–18 hours after the ICSI to approve fertilization (Zygotes) and ensure the presence of two polar bodies and two pronuclei. If the fertilized oocytes had either 1PN or 3PN, they were deemed abnormal. It was considered that oocytes lacking PN were not fertilized. The following formula was utilized to calculate the fertilization rate: The calculation of fertilization rate is (total number of injected MII retrieved oocytes / number of fertilized oocytes (2PN) times 100 (Magaton et al., 2023).

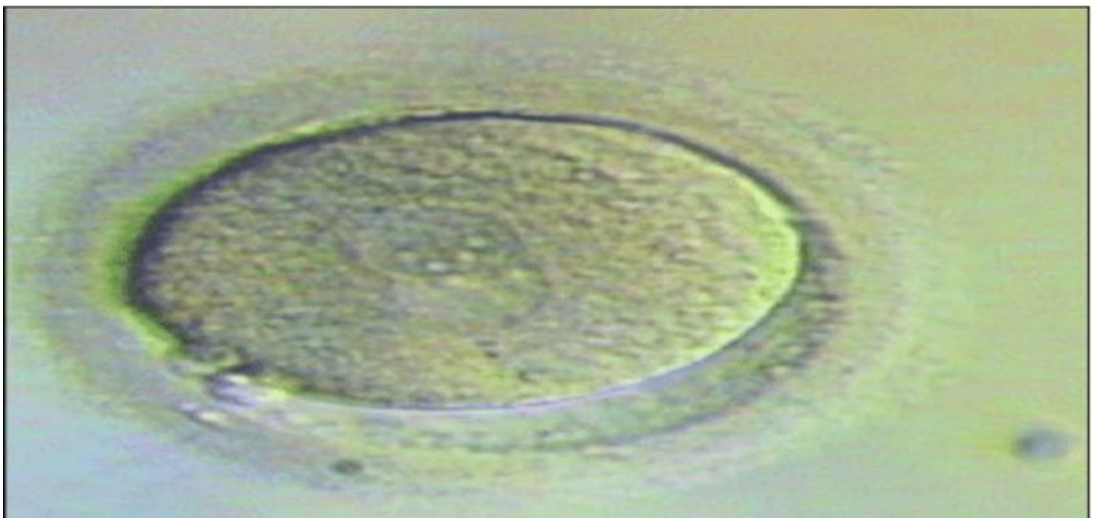


Figure 3.6. Fertilized oocyte (appearance of two pronuclei) is an indicator of fertilization (Merchant et al. 2011)

First-day cleavage was assessed 24 hours after fertilization, and the number and size of blastomeres in each embryo were counted. On days 2 and 3 (48 and 72 hours after oocyte harvest), the amount, size, symmetry, and percentage of blastomere fragmentation determine how the embryos are graded. As seen in Figures 3.7–3.9, the developing embryos were morphologically graded according to the Istanbul consensus workshop prior to embryo transfer, where the embryo was classified into grades I, II, and III (Balaban et al., 2011).

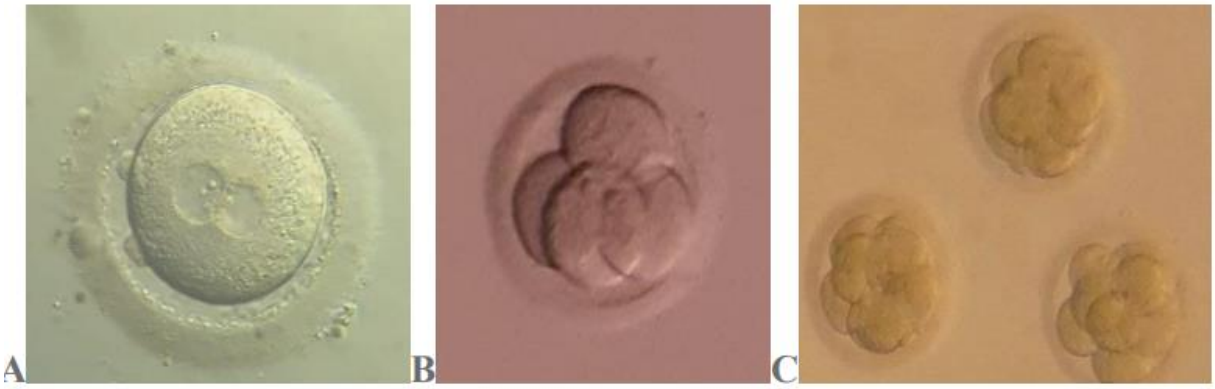


Figure (3-7): Stages of human pre-implantation embryo development. Phase-contrast images of human embryo development from day (d) 0 to day 3. Following fertilization, embryos undergo a series of mitotic cell divisions. A: Zygote, B: 4 cells Grade 1, C: 8cells Grade 1(High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Iraq, Baghdad, 2023).

Grade II: High quality, most cells have stage-specific cell sizes, even in cell division embryos with 10–25% fragmentation.

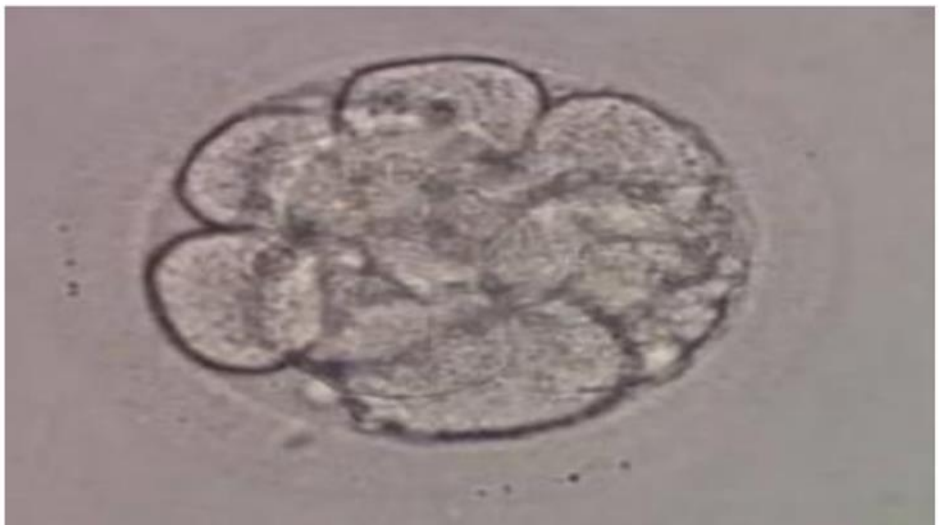


Figure 3.8: Adapted from the High Institute for Infertility diagnosis and Assisted Reproductive Technologies, Al Nahrain University, Baghdad, Iraq; Day 3 grade 2 embryo 8 cells, equal size, 20% fragmentation

Grade III: Embryos of fair quality that show signs of multinucleation, fragmentation (>25%), and uneven cell division.

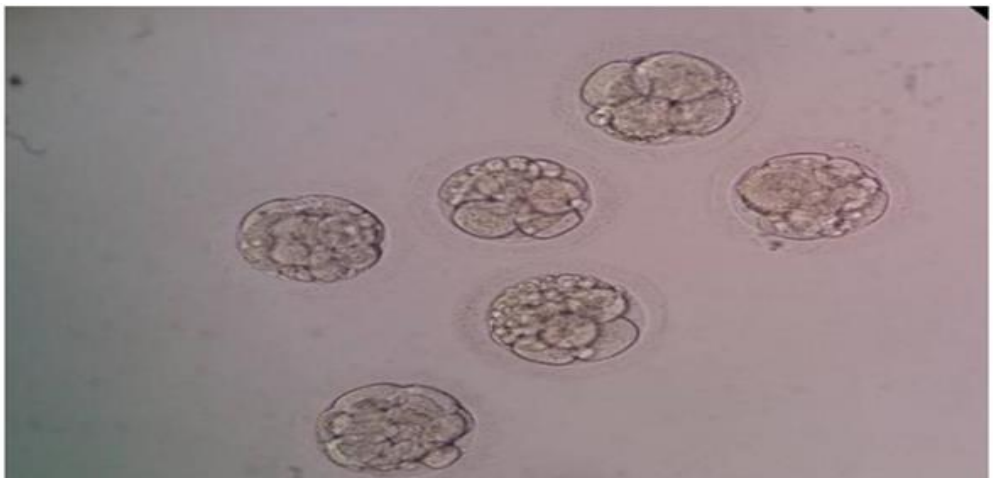


Figure 3.9: Adapted from the High Institute for Infertility diagnosis and Assisted Reproductive Technologies, Al Nahrain University, Baghdad, Iraq, Day 3 grade 3 embryos (5–6) cells, uneven, >25% fragmentation.

3.3.4.6. Embryo Transfer

Anesthesia was not necessary for the embryo transfer procedure (Orvieto et al., 2009). Based on the patient's age, clinical history, number of prior tries, and embryonic quality, the number of embryos selected for transfer was determined (Kamath et al.,2020). Using a flexible catheter (Gynetics®, Belgium) that goes through the cervix and the vagina to enter the uterine cavity, fresh embryo transfer was performed. The patient was positioned in the lithotomy posture with a partially full bladder. To improve the outcome, the cervical mucus was removed and the vagina was gently cleaned with normal saline while being guided by trans-abdominal ultrasound. The highest morphological standards were used in the selection of the transferred embryos. The embryologist loaded and deposited the embryo into the transfer catheter, which was carefully removed after the embryos were transferred to the uterus. The embryo was then examined under a microscope to ensure that every embryo had been transplanted. Typically, the embryo transfer process takes a few minutes. About 14 days following the embryo transfer, a pregnancy test was conducted to ensure the pregnancy was successful.

3.3.4.7. Luteal Phase Support

The luteal phase was maintained starting from oocyte retrieval day through the administration of vaginal progesterone (Cyclogest®400mg twice daily; Actavis, UK®) and progesterone injection (depote 250-500 mg twice weekly) for a duration of 14 days. This regimen continued until the β-HCG test was conducted; in case of a positive result, progesterone intake was sustained until 9-12 weeks, coinciding with the initiation of placental activity (Zhang et al. 2020).

3.3.5. Samples collection

3.3.5.1. Blood Sampling

` Blood Sampling for TNF-α & IL-10, on the day of OPU and day of embryo transfer for both hCG group together with control group and according to the number and grading of the embryos, patients were prepared for the transfer. The patient's serum was obtained by aspirating the blood samples from the median cubital vein by disposable syringe (Becton Dickinson company, USA), into a serum separating tube (gel & clot activator, AFCO, Jordan), and allowed to clot for 30 minutes. Serum was obtained after centrifugation at a rate of 5000 rpm for 5 minutes. All samples were kept in a sterile container labeled with patient's study number. Samples were refrigerated at -20°C until the time of examination using diagnostic Kit by (ELIZA) technique (IL-10, TNF, ELIZA Kit, Bio Source, USA).

3.3.6. Biochemical Assay

3.3.6.1. Principle of the ELISA:

Enzyme-linked immunosorbent assay (ELISA) is a biochemical assay that uses antibodies and an enzyme-mediated color change to detect the presence of either antigen such as proteins, peptides, and hormones or antibody in a given sample (Gan SD.et al., 2013), Figure (3-10)

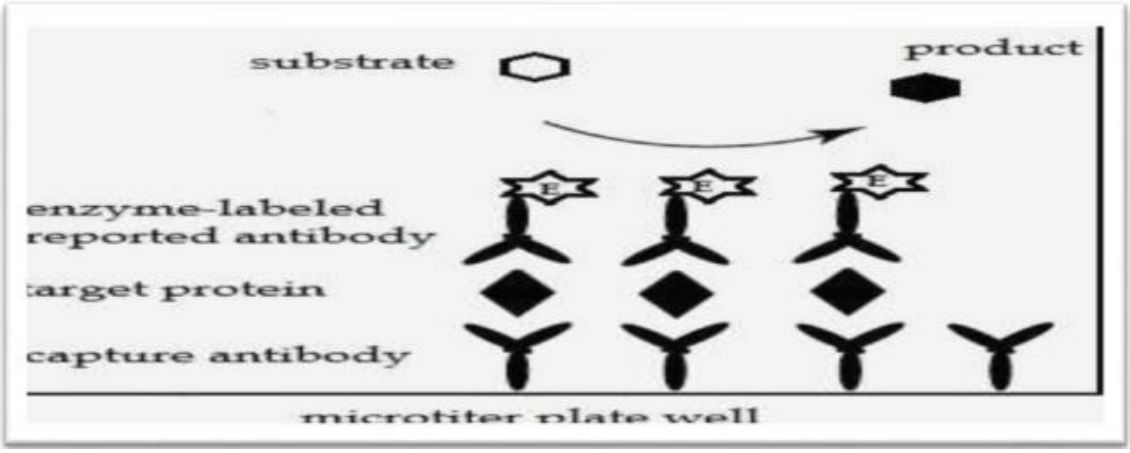


Figure (3-10): Enzyme-linked Immunosorbent Assay (ELISA) (Shan et al., 2002

3.3.6.2. TEST PRINCIPLE FOR IL-10

This kit uses enzyme-linked immune sorbent assay (ELISA) based on the Biotin double antibody sandwich technology to assay the Human Interleukin 10 (IL-10). Add Interleukin10 (IL-10) to the wells, which are pre-coated with Interleukin 10(IL-10) monoclonal antibody and then incubate. After that, add anti IL-10 antibodies labeled with biotin to unite with streptavidin-HRP, which forms immune complex. Remove unbound enzymes after incubation and washing. Add substrate A and B. Then the solution will turn blue and change into yellow with the effect of acid. The shades of solution and the concentration of Human Interleukin 10 (IL-10) are positively correlated figure (3-11).

A **B**

Figure (3-11): ELIZA washer and shaker

3.3.6.3. TEST PRINCIPLE FOR TNF-α

This kit uses enzyme-linked immune sorbent assay (ELISA) based on the Biotin double antibody sandwich technology to assay the Human Tumor necrosis factor-alpha (TNF-alpha). Add Tumor necrosis factor-alpha (TNF-alpha) to the wells, which are pre-coated with Tumor necrosis factor-alpha (TNF-alpha) monoclonal antibody and then incubate. After that, add anti TNF-alpha antibodies labeled with biotin to unite with streptavidin-HRP, which forms immune complex. Remove unbound enzymes after incubation and washing. Add substrate A and B. Then the solution will turn blue and change into yellow with the effect of acid. The shades of solution and the concentration of Human Tumor necrosis factor-alpha (TNF-alpha) are positively correlated.

3.3.6.4. Assay procedure for TNF-alpha:

- 100 µL standard or sample to each well will be added. Incubate for 90 min at 37°C.
- The liquid will be removed. 100 µL will be added Biotinylated Detection Ab. Incubate for 1 hour at 37°C.
- Aspiration and wash 3 times.

- 100 µL HRP Conjugate working solution will be added. Incubate for 30 min at 37°C.
- Aspiration and wash 5 times.
- 90 µL Substrate Reagent will be added. Incubate for 15 min at 37°C.
- 50 µL Stop Solution will be added. Read at 450 nm immediately.
- Results will be Calculated.

3.3.7. Ultrasonography Assessment:

3.3.7.1. Endometrial Thickness Measurement

The transvaginal scan was performed using the 5-9 MHz vaginal probe of SonoAceX6 Ultrasound Set, wall filters (50 Hz) were used to eliminate low frequency signals, Ultrasound machine was performed after placing patients with an empty bladder in the dorsal lithotomy position, a sweep through the mid-sagittal plane of the uterus was obtained to give a multiplanar display figure (3-12).

Figure 3.12: Versana Balance (ge-medical system) ultrasound equipment, CHINA, JIANGSU CHINA (High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Iraq, Baghdad, 2022).

Endometrial thickness of the uterus was measured, as the maximum distance from one basal endometrial interface across the endometrial canal to the opposite endometrial– myometrial interface, after the patient had completely emptied her bladder. The measurement involved both endometrial layers excluding the surrounding low amplitude echo layer, three measurements were taken & the average value was recorded figure (3-10).

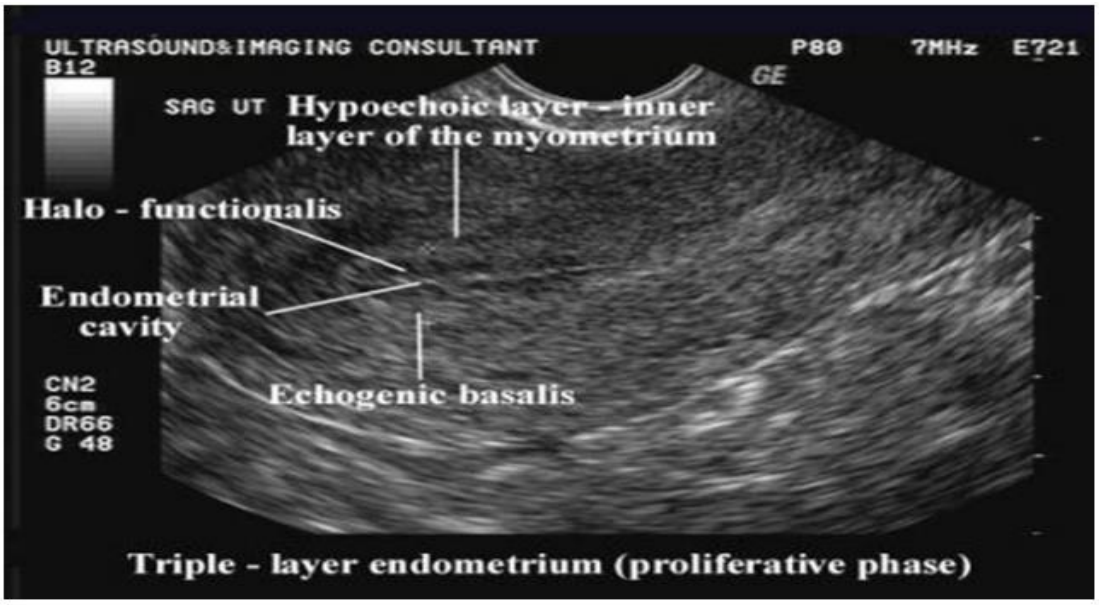


Figure 3.13: Ultrasound review reveals myometrial, endometrial and subendometrial zones (Zhao et al., 2012)

3.3.7.2. Two-Dimensional color doppler Ultrasound

After completion of the B-mode examination, a pulsed Doppler system was used for blood flow analysis. Sub endometrial vessels were visualized at the endometrial periphery, sometimes penetrating the hyperechogenic endometrial edge or even reaching the endometrial cavity (Elnaggar et al., 2017), The blood flow velocity waveforms from the subendometrial vessels were obtained by placing the Doppler gate over the colored area at zone 2 and activating the pulsed Doppler function. Figure (3-14)

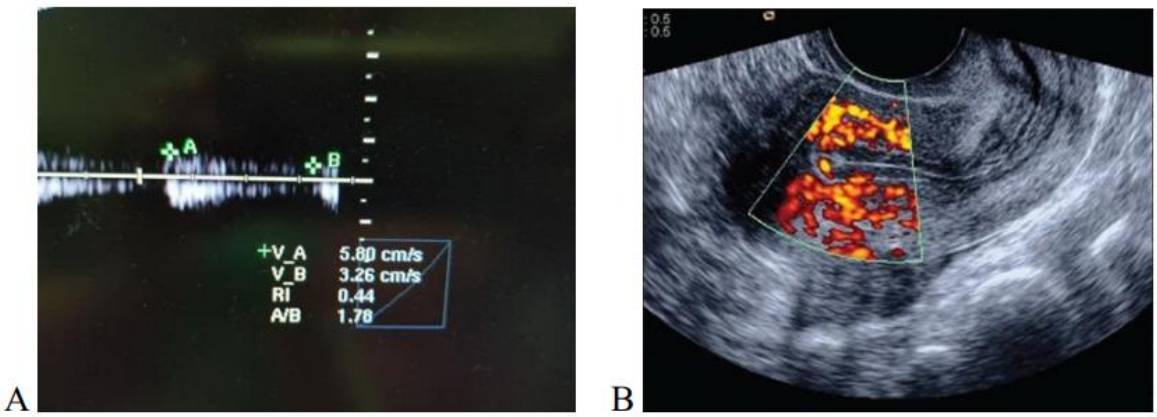


Figure 3.14: A: Subendometrial blood vessels Doppler ultrasound velocimetry in a case of good receptive endometrium. B: Doppler blood flow till Zone 3

A recording was considered satisfactory when at least 3 consecutive uniform waveforms were obtained; each demonstrated the maximum Doppler shift. Three measurements for each parameter were taken and the average value was recorded, Figure (3-12). Resistance index (RI) and pulsatility index (PI) were calculated automatically by ultrasound for both subendometrial vessels, according to the following formula figure (3-15):

Figure 3.15: three consecutive uniform waveform obtained from doppler study of patient attend high institute of infertility diagnosis and assisted reproductive technology.

$$\mathbf{RI} = \frac{\mathbf{PSV-EDV}}{\mathbf{PSV}} \qquad \mathbf{PI} = \frac{\mathbf{PSV-EDV}}{\mathbf{mv}} \qquad \mathbf{mv} = \frac{\mathbf{PSV+EDV}}{\mathbf{2}}$$

RI: resistance index, PSV: peak systolic velocity, EDV: end-diastolic velocity, PI: pulsatility index, mv: mean velocity

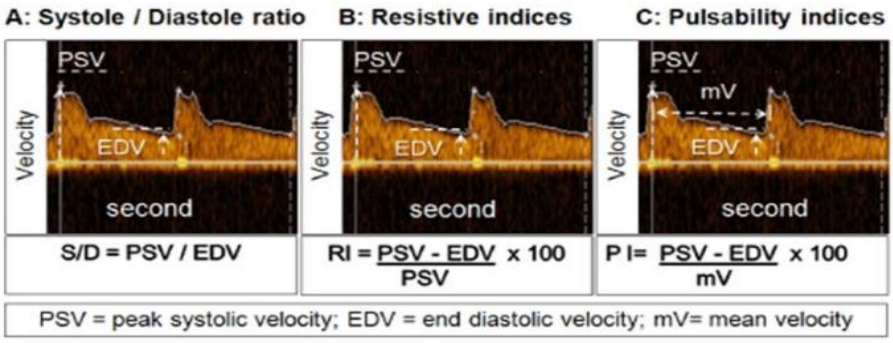


Figure 3.16: Method of measurement of S/D, RI& PI

3.3.8. Pregnancy Test (P.T)

Pregnancy is documented by an elevated serum β-hCG level of ≥ 6.5 mIU/l in the 14th day following embryo transfer, and Progesterone treatment was continued up to 12 gestational weeks (Vaisbuch et al., 2012). Ultrasound examination is performed 6th week after E.T in order to confirm clinical pregnancy, viability and for diagnosis of multiplicity.

3.3.9. HCG-Intrauterine Infusion

HCG-intrauterine infusion done for 60 women undergoing ICSI to improve endometrial receptivity. Two different doses of hcg (diclair ^{R})1500 IU were used for each patient. Drug was used on day of ova pick up. In the study group, the patient was put in the lithotomy position, and the cervix was visualized using Cusco's speculum. The cervical mucous was wiped out using a sterile piece of gauze, then the mucous was partially removed by gentle suction with a 1-mL syringe. A dummy ET was performed using a soft catheter (Wallace-Smiths Medical International Ltd.); if the soft catheter did not easily pass the internal

cervical os, another more rigid but malleable catheter (Cook IVF) was used. After the catheter had passed the internal cervical os.

3.3.9.1. Preparation of hCG

HCG dose was prepared by using , 1 ml of tissue culture media (G.2 plus ref. 10132, Vitrolife) to one vial containing 1500 IU of hCG (Diclair®). The injection dissolved immediately after completion of ova pick up and infused directly to endometrial cavity. 35 µL of tissue culture medium containing 500, or 47 µL of tissue culture media containing 700 IU of hCG was injected intrauterine. Dose prepared by using (1 ml) of gain medium to dissolve hcg injection and dose for each patient loaded by using IUI (Gyntics) catheter.

3.3.9.2. GAIN TM medium

Single-step culture medium for the in vitro culture of human embryos and gametes GAIN™ medium is sterilized by sterile filtration. Medium is a ready to use. Medium is a bicarbonate-buffered balanced salt solution with 10 mg/liter gentamicin and 3.5 g/liter human serum albumin. GAIN™ medium should generally be used in an incubator at 37°C set at 5% CO^2 at normal atmospheric pressure

COMPOSITION GAIN™ medium is a bicarbonate-buffered balanced salt solution with 10 mg/liter gentamicin and 3.5 g/liter human serum albumin.