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Improving Endometrial Ultrasound Parameters and Enhancing Implantation in ICSI Patients: The Role of Autologous Platelet-Rich Plasma Intra-Uterine Infusion

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ABSTRACT

Implantation is a complicated process that involves interactions between the endometrium and the blastocyst and involves adhesion molecules, cytokines, growth factors, lipids, and other components. Autologous platelet-rich plasma (PRP) is thought to be able to enhance endometrial development and improve pregnancy outcomes in ICSI patients by releasing granules containing growth factors including TGF-, PDGF, IGF-1, VEGF, and EGF, which drive the inflammatory cascade and healing process. The purpose of this study was to realize how intrauterine infusions of autologous platelet rich plasma (PRP) affected endometrial angiogenesis and vascularity on the day of ovulation trigger in women undergoing ICSI cycles, as measured by transvaginal ultrasonography and Power Doppler. Eighty-one women, all under the age of 40, were subjected to antagonist ovarian stimulation procedures based on their age, medical history, physical examination, and basal hormone level, and then oocytes were extracted using an ultrasound guidance and fertilized by sperm injection into the egg (Intra Cytoplasmic Sperm Injection). Forty-four of them (PRP group) received intrauterine autologous platelets rich plasma infusion produced from an individual's entire blood then centrifuged to remove red blood cells on the day of ovulation trigger, while the other 37 women did not (Non-PRP group). On the day of ovum pickup, all patients had their ultrasounds taken. In two-dimensional mode, power Doppler is used, electronically determined endometrial thickness, pulsatility index (PI), and resistance index (RI) of sub endometrial arteries. With highly significant deference between PRP and Non-PRP group for endometrial thickness =10.38 ±1.56 versus 9.31 ±1.36, respectively, P = 0.002, and resistance index = 0.49 ±0.08 versus 0.66 ±0.08, and pulsatility index= 0.78 ±0.55 versus 1.04 ±0.20, P = 0.009, respectively, the pregnancy rate in all enrolled infertile women was 18 out of 81 (22.2 %). The rate was higher in PRP group in comparison with Non-PRP group, 31.8 % versus 10.8 %, respectively and the difference was significant (p = 0.023). Autologous PRP intrauterine infusion on the day of ovulation trigger can improve implantation and clinical pregnancy rates in ICSI patients, according to this study. Ultrasonographical, sub-endometrial measures such as endometrial thickness, RI, and PI changed after PRP infusion, as

the thickness increased while vascular resistance reduced, and were all used to predict endometrial receptivity.

1. INTRODUCTION

Implantation is a complicated process that involves interactions between the endometrium and the blastocyst and involves adhesion molecules, cytokines, growth factors, lipids, and other components. The endometrial-myometrial junction (EMJ) is a transitional zone between the endometrium's mucous membrane and the myometrium's outer smooth muscle layer. The uterine junctional zone refers to the inner layer of myometrium that surrounds the endometrium [6]. The endometrium undergoes morphological, cytoskeletal, biochemical, and genetic modifications to become functionally competent throughout the receptivity phase. The "window of implantation" is the term for this period of receptivity (WOI). When Progesterone reaches peak serum concentrations, the WOI opens on day 19 or 20 of the cycle and remains open for only 4–5 days. The commencement of conversation between the free-floating blastocyst and the receptive endometrium, which is mediated by locally active hormones and growth factors, is the first stage towards implantation [20]. The uterine environment is favorable to blastocyst implantation during the window of implantation (WOI). Approximately two-thirds of implantation failures are caused by insufficient uterine receptivity, while the embryo is responsible for just one-third of these failures. The endometrial vasculature's remodeling and development is crucial for embryonic growth and survival [2]. Platelets contain about 30 bioactive proteins, several of which are critical for hemostasis and tissue repair. All wound healing is initiated by seven key protein growth factors that are actively released by platelets, which alter shape and generate pseudopodia when activated (e.g., by thrombin), and promotes platelet aggregation and subsequent release of granule content through the open canalicular system [8]. The growth factors in the residual plasma are 5- to 10-fold higher than in whole blood. The stimulation for endothelial cell proliferation and angiogenesis, according to the scientists, peaks at 1.25×10^6 platelets/mL and 1.5×10^6 platelets/mL, respectively. This indicates that a PRP platelet count of 1 million/mL has become the working standard for therapeutic PRP, and it also explains why some people have complained about not getting the optimal outcomes from PRP, which could be related to lower concentrations of platelets [5].

Ultrasound is now an important part of the current examination of female infertility and assisted conception management. Endometrial ultrasound testing is a frequent non-invasive approach for determining endometrial receptivity during in vitro fertilization (IVF) treatment. Because a strong blood supply to the endometrium is widely thought to be a prerequisite for implantation, monitoring endometrial blood flow during IVF treatment has gotten a lot of attention in recent years [13]. Ultrasound measurements of the endometrium on the day of ovulatory trigger and embryo transfer day (the first interaction between embryo(s) and the uterine environment) may provide a window into the developing egg and the implantation environment [7]. The presence of arteries within the endometrium is linked to a thicker endometrium, suggesting that endometrial perfusion and endometrial growth are linked. The absence of endometrial-sub endometrial blood flow, on the other hand, is accompanied by a high uterine artery resistance [10]. In IVF cycles, the resistance index (RI) evaluated on the day of embryo transfer was much lower in patients who went on to become pregnant than in those who did not. If the pulsatility index (PI) is normal or low, the number of embryos transferred may be lowered to lessen the chance of multiple pregnancy. It is generally agreed that a hypoechogenic endometrium with a thickness of ≥ 7 mm and low RI

and PI, on the day of hCG corresponds to a receptive endometrium [12], [17].

2. 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 October 2019 to April 2021. Eighty-one Iraqi women were included in this study. Every patient gave her written informed consent before taking part in the study, which was approved by the Ethics Committee, Al-Nahrain University. They were evaluated twice during the study period and they did not receive any monetary compensation for their participation. Total of eighty-one patients undergoing IVF/ICSI cycle were evaluated. One out of three types of controlled ovarian hyperstimulation (COH) protocols had been chosen for all patients, which is the antagonist protocol. Follow up of patients done with serial vaginal ultrasound and serum level of estradiol, then accordingly ovum pick up done. At day of Trigger of ovulation, 2D Power Doppler ultrasound of endometrium-zone 2, forty-four women undergoing *in vitro* fertilization (IVF) had been undergone intrauterine infusion of 0.5-1 ml of autologous PRP. At day of oocyte retrieval, again, 2D Power Doppler ultrasound of endometrium-zone 2 measured for all patients, Trans vaginal ultrasound guided Oocyte retrieval done after triggering of ovulation with hCG about 35-36 hrs. At the day of embryo transfer, usually 2 or 3 days according to the number and grading of embryos, embryo transfer done for all. The dosage of rFSH was adjusted according to the ovarian response, which was assessed by ultrasound and serum E2 levels. The time interval from trigger to oocyte pickup (OPU) is vital, which consists of the luteinization start, the cumulus cell expansion, and the reduction division of the oocyte resumption. In most in vitro fertilization (IVF) cycles, the commonly practiced interval was 34–36 h.

Later on, all oocytes underwent denudation and grading at Laminar Flow Cabinet. After insemination, zygotes were observed for 18 - 20 hours to check for the presence of two pronuclei and 25 - 29 hours to confirm the existence of early cleavage, which was correlated with higher implantation rates. Within the embryonic cohort, we transferred embryos with the best kinetic and morphologic criteria. Compacted embryos were given the priority for transfer, whether or not originated from good quality embryos. The transvaginal scan was performed. Endometrial thickness was measured in the median longitudinal plane of the uterus, 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, and endometrial measurement was repeated two times. Endometrial morphology was classified as types A (hyperechoic), B (isoechoic) and C (triple-line) endometrium, respectively. A triple-line pattern was described as hypo-echogenic endometrium surrounded by a hyper-echogenic zone, the junctional zone is hypoechoic due to decreased water content, and is formed by smooth muscle cells that are tightly packed. The extracellular matrix and water content are sparse. It usually measures less than 8mm, figure (1).

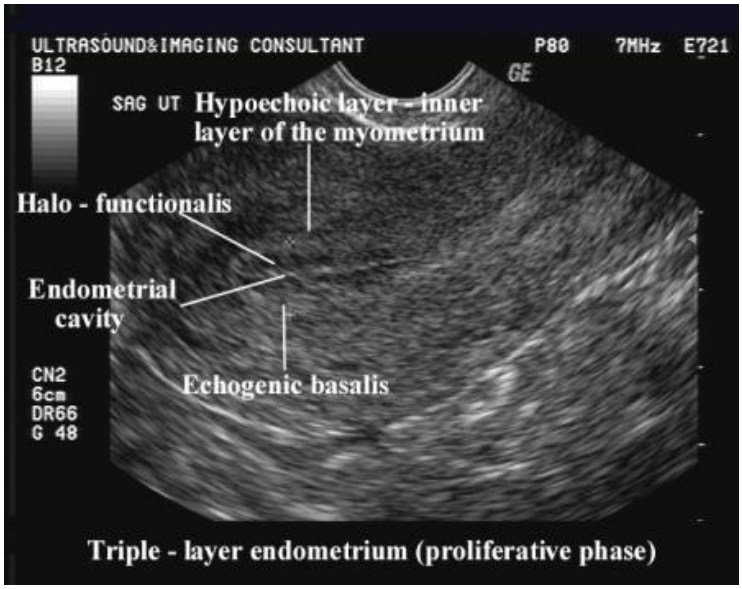

Figure (1): Ultrasound review reveals myometrial, endometrial and sub endometrial zones. (Pin by Gtouchantzidis on uterus ...pinterest.com)

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 & the average value was recorded. figure (2). Resistance index (RI) and pulsatility index (PI) were calculated automatically by ultrasound for both subendometrial vessels, according to the following formula:

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

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

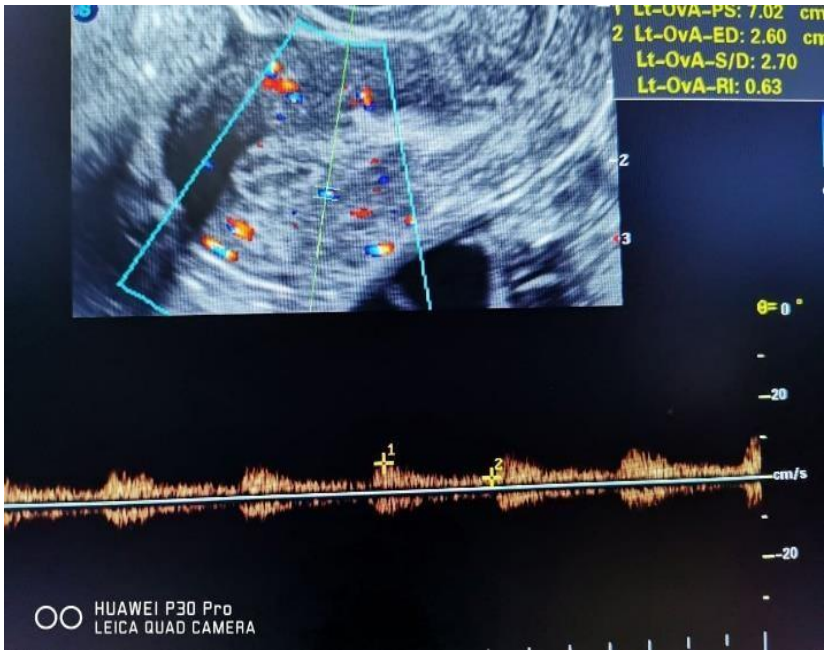

Figure (2): Sub endometrial blood vessels Color Doppler ultrasound velocimetry with pulsed type in a case of good receptive endometrium (High Institute of Infertility Diagnosis and ARTs /Al-Nahrain University, IRAQ, Baghdad).

2.1 Preparation of Platelet Rich Plasma (PRP)

Autologous PRP is obtained from a samples of patients' venous blood drawn at the day of hCG trigger for study cases only, 30-cc venous blood draw will yield 3-5 cc of PRP, so 5-6 cc of blood is fair enough to yield 0.5-1 ml PRP, depending on the baseline platelet count of an individual, the device used, and the technique employed. The blood draw occurs with the addition of an anticoagulant, such as citrate dextrose A, to prevent platelet activation prior to its use.

2.2 Principles of PRP Preparation

After obtaining written informed consent from the selected women, PRP is prepared by a process known as differential centrifugation, by which acceleration force is adjusted to sediment certain cellular constituents based on different specific gravity. PRP is prepared by centrifugation varying the relative centrifugal force, temperature and time. There are many ways of preparing PRP. It can be prepared by the PRP method or by the buffy-coat method, we used to do it by Buffy coat method. Peripheral hole blood (WB) was collected using tubes containing 3.2% sodium citrate solution and should be stored at 20°C to 24°C before centrifugation. WB was Centrifuged at a 'high' speed (3000 g for 6 min.). Three layers are formed because of its density: The bottom layer consisting of RBCs, the middle thin layer (buffy coat) consisting of platelets and WBCs and the top PPP layer. The difficulty lies in separating this thin buffy coat layer from the underlying RBC layer. Supernatant plasma (PPP) was Removed from the top of the container. Buffy-coat layer was Transferd to another sterile tube. Centrifugation was done at low speed to separate WBCs (2800 g for 3 min.). About 0.4-0.5 ml of PRP pushed through intrauterine insemination catheter into the uterine cavity while the patient lying in lithotomy position and after washing of vulva and vagina with normal saline. This step usually preceded by ultrasound examination of endometrium (zone-2).

3. Results

3.1 Demographic characteristics of infertile women

The demographic characteristics of infertile women enrolled in the current study are shown in table (1). There was no significant difference in mean age between PRP group and Non-PRP group (p = 0.394) but there was significant difference in mean duration of infertility between PRP group and Non-PRP group (p = 0.039). there was no significant difference in mean BMI between PRP group and Non-PRP group (p = 0.450). and there was no significant difference in the frequency distribution of infertile women according to type of infertility between PRP group and Non-PRP group (p = 0.519).

Table (1): Comparison of demographic parameters between PRP a Non-PRP ICSI groups of patients

Characteristic	Total n = 81	PRP group n = 44	Non-PRP group n = 37	p
Age (years)				
Mean ±SD	29.44 ±5.87	28.93 ±5.63	30.05 ±6.16	0.394 I NS
Range	16 -40	18 -40	16 -40	

Duration of infertility (years)				
Mean ±SD	7.03 ±3.65	7.80 ±3.92	6.12 ±3.11	**0.039 I S**
Range	1 -17	1 -17	2.5 -16	
BMI (kg/m^2)				
Mean ±SD	27.05 ±3.42	27.31 ±3.75	26.73 ±3.02	0.450 I NS
Range	20.2 -36.7	20.2 -36.7	20.42 -30.67	
Type of infertility				
Primary, *n* (%)	68 (84.0 %)	38 (86.4 %)	30 (81.1 %)	0.519 C NS
Secondary, *n* (%)	13 (16.0 %)	6 (13.6 %)	7 (18.9 %)	

n: number of cases; SD: standard deviation; BMI: body mass index; I: independent samples t-test; C: chi-square test; NS: not significant at $p > 0.05$

3.2 Serum hormonal levels at cycle day two of infertile women

The serum hormonal levels of infertile women enrolled in the present study are shown in table (2); there was no significant difference in mean FSH between PRP group and Non-PRP group ($p = 0.155$). there was no significant difference in mean LH between PRP group and Non-PRP group ($p = 0.747$). there was no significant difference in mean estradiol between PRP and Non-PRP group ($p = 0.894$). there was no significant difference in mean prolactin between PRP group and Non-PRP group ($p = 0.149$). there was no significant difference in mean TSH between PRP group and Non-PRP group ($p = 0.884$).

Table (2): Comparison of hormonal profile between PRP and Non-PRP ICSI groups of patients

Characteristic	Total *n* = 81	PRP group *n* = 44	Non-PRP group *n* = 37	*p*
FSH (mIU/ml)				
Mean ±SD	7.18 ±2.19	6.86 ±1.90	7.56 ±2.46	0.155 I NS
Range	0.25 -17	3.15 -10.7	0.25 -17	
LH (mIU/ml)				
Median (IQR)	5.31 ±1.72	5.26 ±1.51	5.38 ±1.95	0.747 I NS
Range	2.7 -10.4	3.08 -10.4	2.7 -9	
Estradiol (pg/ml)				
Mean ±SD	33.52 ±12.57	33.70 ±13.05	33.32 ±12.16	0.894 I

Range	0.5 -69	4.7 - 69	0.5 -55	NS
Prolactin (ng/ml)				
Mean ±SD	16.65 ±7.90	17.81 ±8.87	15.26 ±6.41	0.149 I NS
Range	1.5 -42	2.5 -42	1.5 -27	
TSH mIU/L				
Mean ±SD	1.97 ±1.17	1.95 ±1.48	1.99 ±0.66	0.884 I NS
Range	0.3 -7.3	0.39 -7.3	0.3 -3.2	

n: number of cases; SD: standard deviation; IQR: inter-quartile range; FSH: follicle stimulating hormone; LH: Luteinizing hormone; TSH: thyroid stimulating hormone; I: independent samples t-test; NS: not significant at $p > 0.05$; HS: highly significant at $p \leq 0.01$

3.3 Ultrasound findings, Doppler biophysical profile in all enrolled women (PRP and Non-PRP group) at day of Ova Pickup

The ultrasound findings, biophysical profile at day of OPU in all enrolled infertile women are shown in table (3), there was highly significant difference in mean endometrial thickness between PRP group and Non-PRP group ($p = 0.002$); the thickness being higher in PRP group.

There was highly significant difference in mean resistance index between PRP group and Non-PRP group ($p < 0.001$); the resisance index being lower in PRP group, there was highly significant difference in mean pulsatility index between PRP group and Non-PRP group ($p = 0.009$); the pulsatility index being lower in PRP group.

Table (3): Ultrasound findings, biophysical at day of OPU in all enrolled infertile women

Characteristic at day of ova pickup	**Total** ***n* = 81**	**PRP group** ***n* = 44**	**Non-PRP group** ***n* = 37**	***p***
Endometrial thickness				
Mean ±SD	9.89 ±1.56	10.38 ±1.56	9.31 ±1.36	**0.002 I HS**
Range	6.40 -13.40	7.00 -13.40	6.40 -12.00	
Resistance index				
Mean ±SD	0.57 ±0.12	0.49 ±0.08	0.66 ±0.08	**< 0.001 I HS**
Range	0.31 -0.80	0.31 -0.72	0.45-0.80	
Pulsatility index				

Mean ±SD	0.91 ±0.44	0.78 ±0.55	1.04 ±0.20	**0.009 I**
Range	0.20 -3.26	0.20 -3.26	0.73-1.23	**HS**

n: number of cases; SD: standard deviation; I: independent samples t-test; S: significant at $p \leq 0.05$; NS: not significant at $p > 0.05$; HS: highly significant at $p \leq 0.01$

4. Discussion

The presence of a viable embryo, a receptive endometrium, proper embryo endometrial cross-talk, and enough mother immune protection are all required for successful implantation. Despite breakthroughs in assisted reproductive technology, implantation and pregnancy rates have not improved significantly. According to demographic and infertility-related characteristics as provided in Table (1), there is no significant differences between PRP group and Non-PRP group except in mean duration of infertility, the mean duration of infertility in Non- PRP group was ranging from 3-9 years, with the mean ± SD (6.12 ±3.11), while in PRP group was ranging from 3-11 years, with the mean ± SD (7.80 ±3.92). Furthermore, male and female patients with infertility for more than three years had lower core and total fertility quality of life and psychological health, as well as lower perceived treatment-related quality of life [21]. As the years of infertility pass, this stress is likely to escalate, potentially exacerbating psychological problems. About hormonal assay at day two of stimulation cycle, in the present study, there was no significant difference in mean E2, FSH, LH, PRL and TSH level at day two of stimulation cycle, between PRP group and Non-PRP group, because all the findings were comparable between the two groups to ensure statistical matching and reduce any variations that could affect the study's outcome. Ultrasound measurement of endometrial thickness is a simple, non-invasive tool that has been investigated as an indicator of endometrial receptivity and a probable predictor of IVF-ET treatment effectiveness. The purpose is to provide a more precise estimation of the potential for implantation [19]. In the present study, there was highly significant difference between both groups at day of OPU, the mean endometrial thicknesses are higher with PRP group. This is supported by [3] who worked hard in 2015 to investigate the efficacy of PRP in the treatment of infertile women with thin endometrium (less than 7 mm). They had five women having IVF with poor endometrial response who still had thin endometrium following normal hormone replacement therapy (HRT) and had to stop the embryo transfer round. In addition to HRT, autologous blood PRP intrauterine infusion was used. In each cycle, PRP infusions were given 1-2 times. When the endometrial thickness reached > 7 mm, embryos were transplanted. Following PRP infusion, all of the patients experienced effective endometrial growth and pregnancy.

At the time, intrauterine PRP infusion was a novel treatment option for thin endometrium with poor response. [9], agreed that the rise in endometrial thickness demonstrated a positive response to PRP therapy. The endometrial pattern was modified from thin linear to triple line after PRP therapy. PRP was found to be effective in reducing sub-endometrial flow and uterine artery resistance, particularly in patients with intrauterine adhesions. -hCG positivity was found in 43.3 percent of their cases. A clinical pregnancy rate of 30% was discovered. They agreed that PRP has potential benefits in patients with suboptimal endometrium by increasing endometrial thickness, improving pattern, and enhancing vascularity with a lower cancellation rate in patients with suboptimal endometrium. Another study, which did not concur with our findings, looked at the use of PRP in patients who had frozen embryo transfers and had normal or optimal endometrial thickness, but found no statistically significant change in pregnancy rates, no significant difference was found between the implantation, clinical pregnancy, ongoing pregnancy, and miscarriage rates of frozen-thawed embryo transfers with and without PRP infusion [1].

At the present study, the mean RI and PI were lower in PRP group, and the difference were highly significant. PRP is a safe, affordable, and acceptable alternative for endometrial preparation of infertile patients with 2 IVF failures due to inadequate endometrium, according to [15]. PRP was injected into the uterus of women with inadequate endometrial receptivity, two IVF failures, and a cancelled HRT cycle. Endometrial vascularity increased in a statistically meaningful way. After PRP therapy, the uterine artery RI and PI decreased statistically significantly, while the implantation rate and clinical pregnancy rate increased [15]. Because platelets contain granules that store growth factors and cytokines, these factors are critical in activating fibroblasts and promoting angiogenesis then vascularization and endometrial receptivity, Mouanness and his colleagues found that PRP was associated with increased stromal and mesenchymal cell proliferation, increased expression of regenerative enzymes, and enhancement in cell migration in in vitro studies [16]. Also, it is important to know that the pregnancy rate in PRP group was higher than that in Non-PRP group of women, this verified the positive effect of autologous PRP intrauterine infusion as a source of all growth factors employed for angiogenesis and endometrial receptivity enhancement, ultrasound sub-endometrial parameters, all, have amazing changes in pregnant group. [4] demonstrated that intrauterine autologous PRP infusion is a safe, low-cost adjuvant treatment for optimizing endometrium, particularly in patients with a history of recurrent implantation failure, and that intrauterine PRP infusion improved not only endometrial lining but also in vitro fertilization success and pregnancy outcome, as well as clinical pregnancy outcome and importantly live birth rate were also significantly higher in PRP group than the control group. Only few writers have refuted the link between intrauterine PRP and clinical pregnancy rates, demonstrating that PRP does not significantly boost implantation rates/clinical pregnancy rates in patients who have previously failed a frozen embryo transfer [11].

5. Conclusions

according to this study, the use of autologous PRP on the day of ovulation trigger can improve implantation and clinical pregnancy rates in ICSI patients. Three features were discovered to be significantly and independently associated to intrauterine PRP infusion. The factors in question include endometrial thickness, RI, and PI.

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