

The relationship between ovarian vascularity and the duration of stimulation in in-vitro fertilization

S.Bassil¹, C.Wyns, D.Toussaint-Demyelle, M.Nisolle, S.Gordts and J.Donnez

In Vitro Fertilization Unit, Department of Gynecology, Catholic University of Louvain, Cliniques Universitaires St Luc, Avenue Hippocrate 10, 1200 Brussels, Belgium

¹To whom correspondence should be addressed

The role of transvaginal pulsed colour Doppler ultrasound in the assessment of ovarian vascularity was studied in 196 in-vitro fertilization (IVF) cycles. The changes in ovarian blood flow after gonadotrophin-releasing hormone agonist (GnRHa) down-regulation and human menopausal gonadotrophin (HMG) stimulation were determined. The data obtained showed that the ovarian blood flow was significantly improved by oestradiol secretion ($P = 0.05$) and human chorionic gonadotrophin (HCG) administration ($P = 0.003$). Folliculogenesis was affected by blood flow supply. The resistance index (RI) value was significantly different ($P = 0.05$) according to the duration of ovarian stimulation. Patients with a mean RI value >0.56 had a longer stimulation with a significantly lower mean number of oocytes retrieved ($P = 0.01$) despite the administration of a standard dose of HMG. The RI value is a good indicator of modifications in ovarian vascularization during stimulation. Doppler blood flow measurement could be used to determine the optimal timing for the beginning of HMG administration in patients undergoing ovarian stimulation after down-regulation for IVF treatment.

Key words: in-vitro fertilization/ovarian stimulation/ovarian vascularity/transvaginal colour Doppler

Introduction

Ovarian haemodynamics during spontaneous (Scholtes *et al.*, 1989; Campbell *et al.*, 1993; Kupesic and Kurjak, 1993) and/or stimulated cycles (Dentinger *et al.*, 1989; Weiner *et al.*, 1993; Balakier and Stronell, 1994) may be responsible for the selection and the maturation of follicles. Previous studies on animals (Zeleznik *et al.*, 1981; Murdoch *et al.*, 1983; Okuda *et al.*, 1983; Köning *et al.*, 1989) have implied that ovarian blood flow may control oocyte ovulation as well as the function of the corpus luteum (Niswender *et al.*, 1976; Damber *et al.*, 1987). In humans, ovarian stimulation increases the ovarian blood flow during the cycle (Dentinger *et al.*, 1989; Balakier and Stronell, 1994) and the haemodynamic variations within the ovary seem to determine the quality of the corpus luteum (Baber *et al.*, 1988).

The combination of transvaginal pulsed colour Doppler

with real time sonography (duplex system) is a non-invasive technique to assess changes in ovarian haemodynamics during stimulation for in-vitro fertilization (IVF).

The use of vaginal sonography provides an image with high resolution and improves the resolution of the flow velocity waveforms. The proximity of the probe to the genital tract facilitates the visualization of the ovarian vessels and decreases the Doppler angle of insonation. As the Doppler beam insonates at a low angle, a large Doppler frequency shift is obtained, which reduces the possibility of error when impedance indices are measured. These impedance indices comprise the resistance index (RI) or the pulsatility index (PI), and were found to be correlated with the size (Balakier and Stronell, 1994) and number (Weiner *et al.*, 1993) of follicles during stimulation.

The aim of our study is to evaluate the haemodynamic modifications in ovarian vascularity during ovarian stimulation and their relationship to the length of stimulation in a standard treatment protocol.

Materials and methods

In a prospective controlled study (between January 1994 and June 1995), 152 patients (196 cycles) treated in our IVF unit were selected for assessment of the bilateral RI of the ovarian arteries. The aetiologies of infertility were male subfertility 67/196 (34%), endometriosis 8/196 (4%), unexplained 21/196 (11%), tubal 86/196 (44%), and mixed 14/196 (7%). The same stimulation protocol was used in all cycles and the exclusion criteria included age >38 years, polycystic ovary disease, periovarian adhesions, ovarian endometriomas, previous major surgery of the pelvis (frozen pelvis, uterine malformation, ovarian wedge resection, ovarian cystectomy, myoma), patients with blood or hypertensive disease and patients using drugs that could influence blood circulation or heart haemodynamics.

Statistical analysis included comparison of RI according to the stimulation parameters, such as the number of oocytes, the serum steroid concentrations and the day of human chorionic gonadotrophin (HCG) administration.

Doppler measurements

A pulsed wave colour Doppler system (Toshiba SSH, 140 high grade, Toshiba, Brussels, Belgium) with a transvaginal transducer of 6.5 MHz and a high-pass filter of 100 Hz was used to obtain flow velocity waveforms. Measurements were carried out by the same operator and the 'gate' of the pulsed Doppler could be positioned in the vessel located on the screen.

Because the visualization of the infundibulo-ovarian vessels is not constant, and in order to standardise measurements, flow velocity waveforms were elicited from a main vessel within the ovarian cortex to avoid any confusion with the ovarian branch of the uterine artery. These measurements reflected the direct modifications in the ovarian vessel dynamics because of the anatomical division of arterial branches

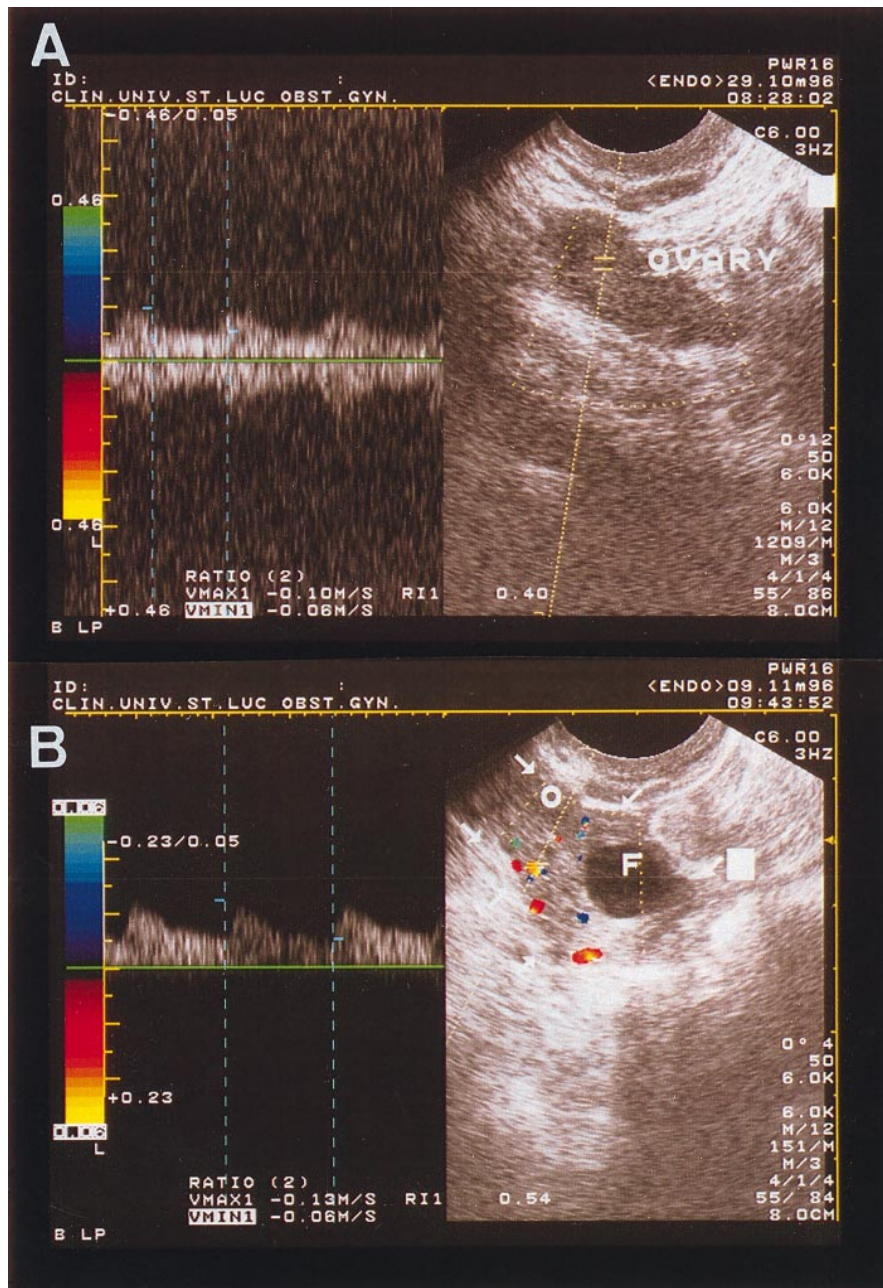


Figure 1. (A) Measurement of the intra-ovarian resistance index (RI) after gonadotrophin-releasing hormone agonist (GnRHa) down-regulation. (B) Measurement of the intra-ovarian RI 6 days after human menopausal gonadotrophin (HMG) administration (arrows show the position of the Doppler window). O = ovary, F = follicle.

within the ovarian hilum. This was clearly demonstrated in animal studies (Köning *et al.*, 1989).

The equation $RI = (A-B)/A$ was used to evaluate the blood flow pattern (where A was the maximum systolic blood flow velocity and B was the end diastolic blood flow velocity) (Figure 1). A recording was considered satisfactory for measurement when there were at least three equally intense waveforms in a row. For the RI assessment, patients were seen 2 days before the start of human menopausal gonadotrophin (HMG) administration, and then on days 7, 9, 11, 13 (the day of HCG administration), 15 (the day of oocyte retrieval) and on the day of embryo transfer. The mean value of the bilateral RI was used for statistical analysis.

Ovarian stimulation and oocyte retrieval

A long protocol with gonadotrophin-releasing hormone agonist (GnRHa) was used for ovarian stimulation in all cycles. All patients received an i.m. injection of triptorelin 3.75 mg (Decapeptyl SR, Lab. Ipsen, France) between days 22 and 25 of the previous cycle. Sonography and oestradiol dosage were carried out 18–23 days after the beginning of GnRHa administration. If the ovaries were at rest and the oestradiol concentration was <30 pg/ml, HMG administration was begun 2 days later in the form of four ampoules on each of the first 4 days, and two ampoules on days 5 and 6. The treatment was adjusted from day 7 according to the number and size of follicles and oestradiol concentrations. HCG was administered (10000 IU)

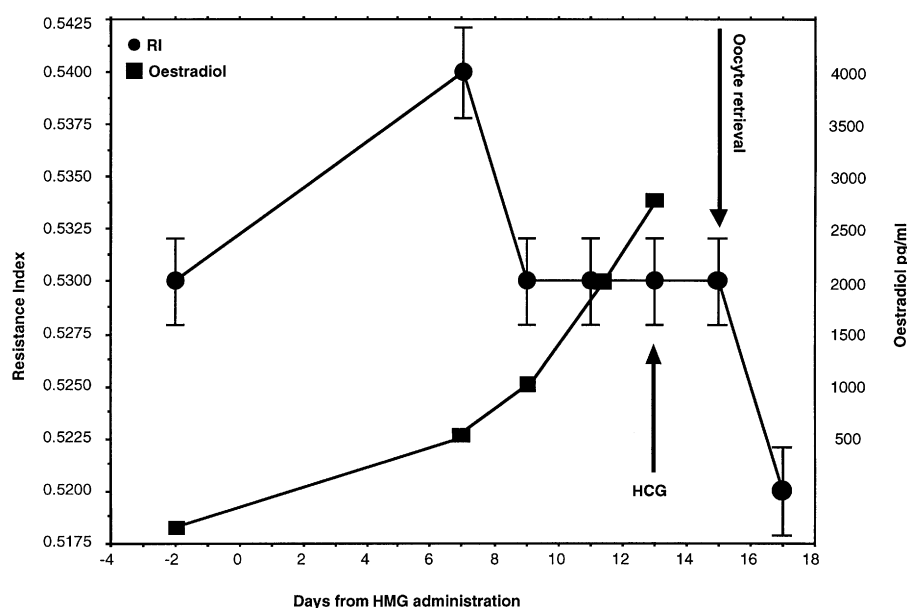


Figure 2. The development of ovarian vascularization according to oestradiol concentration irrespective of duration of stimulation.

when the oestradiol concentration was ~ 200 pg/ml per follicle >16 mm in diameter and in the presence of more than three follicles >20 mm in diameter. Oocyte retrieval was carried out by the vaginal route with sonographic guidance and local anaesthesia.

Statistical analysis

Statistical analysis was carried out using Student's *t*-test and the χ^2 -test. The level of statistical significance was $P \leq 0.05$.

Results

The mean age of patients was 30.7 ± 3.3 years and the mean number of oocytes retrieved per puncture was 15.1 ± 7.4 . The overall fertilization rate was 54%. An embryo replacement was performed in 161 cycles (82%) and 58 pregnancies were achieved from 196 started cycles (29.6% per cycle).

Results of the ovarian vascularization assessment

Figure 2 shows how the ovarian vascularization and oestradiol concentrations changed during the stimulation. The mean RI measurements of the left and right intra-ovarian vessels was used. The days were numbered according to the beginning of HMG administration. The intra-ovarian RI increased from ~ 0.5325 to 0.5400 after the first 6 days of stimulation. This value then decreased to 0.53 within the next 2 days and remained stable throughout the stimulation, despite the increase in oestradiol concentrations. By 96 h after HCG administration, the RI value decreased significantly ($P = 0.003$) to 0.52.

The intra-ovarian RI according to the duration of stimulation

Figure 3 shows the intra-ovarian RI values according to the duration of stimulation. In cycles ($n = 29$) where HCG was administered after the 14th day of stimulation (longer cycles), the mean values of the RI were significantly higher 2 days before ($P = 0.05$) and 6 days after ($P = 0.05$) the beginning

of HMG administration, compared to cycles where HCG was administered on or before the 14th day (shorter cycles, $n = 167$). After the seventh day of stimulation, there was a significant decrease ($P = 0.05$) in the RI value in longer cycles. The intra-ovarian vascularization remained similar in the following days irrespective of when HCG was administered. In longer cycles, a greater decrease of the RI value after HCG administration was observed overall. However, in comparison to short cycle, this decrease was not statistically different.

Stimulation parameters according to the day of HCG administration

Table I shows the distribution of the stimulation parameters according to the duration of stimulation. In cycles with HCG administered after the 14th day of stimulation, the intra-ovarian RI was significantly higher ($P = 0.05$) 2 days before and 6 days after the beginning of HMG administration. Also, the mean number of oocytes retrieved was significantly lower ($P = 0.01$) in those cycles.

Results of the stimulation according to the intra-ovarian RI

In order to compare two groups of patients according to the RI value rather than the duration of stimulation, the distribution of the cycles in relation to the RI value 2 days before (after down-regulation) and 6 days after the beginning of HMG administration (after the standard dose of HMG) was evaluated. The results (Table II) clearly revealed that cycles with an improved ovarian vascularity ($RI < 0.56$) 2 days before the beginning of HMG administration showed a significantly ($P = 0.03$) higher mean number of oocytes retrieved (15.4 ± 7 versus 13.2 ± 6) despite a significantly lower ($P = 0.02$) mean number of ampoules of HMG administered (38 ± 15 versus 43 ± 17). These results were confirmed when we analysed the same parameters according to the RI

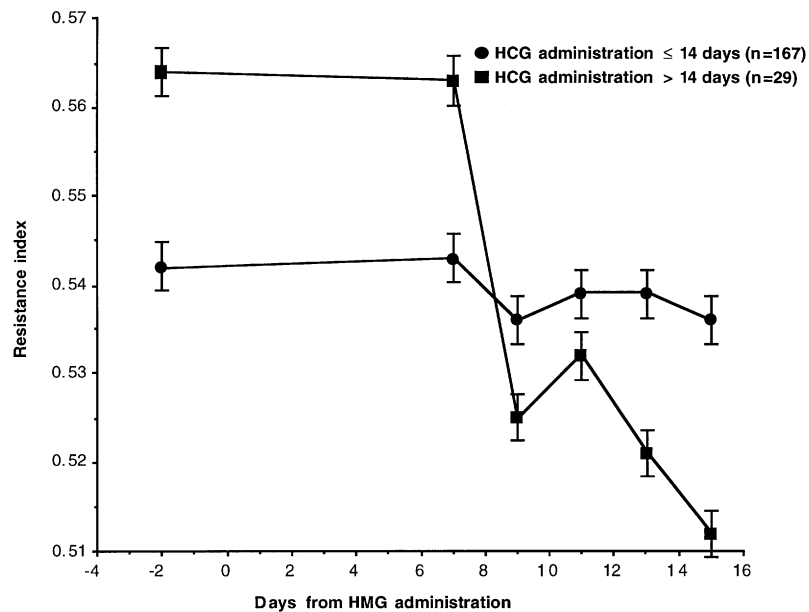


Figure 3. The intra-ovarian resistance index (RI) values according to the duration of stimulation.

Table I. Stimulation parameters according to the day of human chorionic gonadotrophin (HCG) administration

| | HCG administration on or before the 14th day of stimulation ($n = 167$) | HCG administration after the 14th day of stimulation ($n = 29$) | P -value (t -test) |
|---|---|---|-------------------------|
| Age (years) | 30.8 ± 3.4 | 31.5 ± 3.5 | NS |
| No. of oocytes | 14.8 ± 1 | 11.3 ± 6 | 0.01 |
| Oestradiol concentration (pg/ml) on day of HCG administration | 2532.3 ± 944 | 2405 ± 732 | NS |
| Intra-ovarian RI 2 days before the beginning of HMG | 0.542 ± 0.04 | 0.564 ± 0.05 | 0.05 |
| Intra-ovarian RI 6 days after the beginning of HMG | 0.542 ± 0.02 | 0.563 ± 0.03 | 0.05 |

Values are means ± SD.

NS = not significant.

HMG = human menopausal gonadotrophin.

Table II. Cycle distribution according to the ovarian resistance index before and soon after the beginning of stimulation

| | Ovarian RI range | No. of cycles | No. of oocytes (mean ± SD) | Total amp. of HMG used (mean ± SD) |
|-----------------------------|------------------|---------------|----------------------------|------------------------------------|
| 2 days before beginning HMG | <0.56 | 97 | 15.4 ± 7 ^a | 38 ± 15 ^b |
| | ≥0.56 | 99 | 13.2 ± 6 ^a | 43 ± 17 ^b |
| 6 days before beginning HMG | <0.56 | 106 | 15.6 ± 7 | 37.6 ± 14 ^c |
| | ≥0.56 | 90 | 12.8 ± 6 | 44.3 ± 18 ^c |

Values with the same superscript were significantly different by paired

Student's t -test: ^a $P = 0.03$; ^b $P = 0.02$; ^c $P = 0.04$.

HMG = human menopausal gonadotrophin.

values 6 days after the beginning of HMG administration. In cycles with a low RI value, the number of oocytes retrieved was higher compared to cycles with $RI \geq 0.56$ (15.6 ± 7 versus 12.8 ± 6 ; not significant) despite a significantly ($P = 0.04$) lower mean number of ampoules of HMG administered (37.6 ± 14 versus 44.3 ± 18).

Discussion

Taylor *et al.* (1985) were the first to point out the possibility of investigating normal ovarian and uterine blood flow by means of a Doppler system. With the introduction of high frequency transvaginal sonography with colour Doppler (duplex system), measurements of ovarian flow can be obtained with a small angle of insonation. Because of difficulties in properly identifying the ovarian artery, mapping of intra-ovarian vessels is more accurate and provides precise measurements of the RI.

In our study, all measurements were carried out by the same operator with the same parameters of insonation. There was no need to use an angle correction for the RI evaluation. In these conditions, we intended to eliminate the between-observer and machine variations.

At 2–3 weeks after down-regulation with GnRHa (mean 19 ± 4 days; mean oestradiol concentration 19.4 ± 7 pg/ml), the intra-ovarian RI showed a mean value of 0.53 which increased during the fourth week, and this despite the beginning of HMG injections and oestradiol secretion. This is different

from the values found during natural cycles (Scholtes *et al.*, 1989; Kupesic and Kurjak, 1993). The difference could be an effect of the time of exposure to GnRHa or a direct effect of the GnRHa on ovarian vessels, and may explain the high level of oestradiol needed (500 pg/ml) before an increase in the ovarian blood flow was observed. In fact, a direct inhibitory action of GnRHa on both uterine contractility and on oestradiol-induced enzymes involved in cell proliferation has been reported in animal studies (Reddy *et al.*, 1985, Medeiros *et al.*, 1988). Van de Sandt *et al.* (1995) also studied the effect of GnRHa on the uterine blood flow in women with uterine myomas. They observed, in three out of four groups, an increase in the vascular resistance of the uterine perfusion. After day 7, the RI value did not change until the HCG administration, which induced a significant increase in the ovarian vascularity with a decrease in the RI value ($P = 0.003$). This is in accordance with the modifications observed during natural cycles (Scholtes *et al.*, 1989; Campbell *et al.*, 1993; Kupesic and Kurjak, 1993) but does not agree with the results of a recent report by Tekay *et al.* (1995) in which no changes in the Doppler measurements of the intra-ovarian blood flow were found during stimulation. This discrepancy may be due to the site of Doppler measurements and the type of GnRHa used. Tekay *et al.* (1995) achieved down-regulation using buserelin acetate administered intra-nasally, and focused their measurements on the perifollicular vessels. In our study, we used a sustained-release form of GnRHa (triptorelin 3.75 mg) and we focused our measurements on a main vessel within the ovarian cortex avoiding the perifollicular vessels. Folliculogenesis seems to be affected by blood flow supply. This phenomenon was clearly demonstrated by Balakier and Stronell (1994) and by Weiner *et al.* (1993) in stimulated cycles. In our study, Figure 3 and Table I show the relationship between ovarian vascularity and the stimulation outcome. Although the number of cases is small, in the group of cycles where the duration of stimulation exceeded 14 days ($n = 29$), the RI values after down-regulation and 6 days after the beginning of HMG administration were significantly higher ($P = 0.05$) than those observed in cycles ($n = 167$) where the HCG was administered on or before the 14th day of stimulation. Also, in the first group, the mean number of oocytes retrieved was significantly lower ($P = 0.01$) and this despite a similar age in the two groups. In our study, the total dose of HMG administered in the first 6 days was the same in all patients, and the discrepancy in the stimulation response may be related to the individual sensitivity of each woman to the GnRHa. The RI value seems to reflect this sensitivity. These results suggest, but do not prove, that follicular selection may result in preferential vascular delivery of gonadotrophic hormones or other growth factors or proteins required for follicular recruitment and maturation. This hypothesis is supported by the results of histomorphometric, autoradiographic and macroscopic studies on animal ovaries (Zeleznik *et al.*, 1981; Koning *et al.*, 1989; Tanaka *et al.*, 1989). In addition, a physiological relationship between follicular blood flow velocity and oocyte recovery was recently reported (Nargund *et al.*, 1996, Oyensaya *et al.*, 1996). On the other hand, our results show that patients with a higher RI (>0.56) after

GnRHa desensitization have a significantly lower mean number of follicles and need a significantly higher number of ampoules of HMG when compared to patients with a lower RI (<0.56). In our study, the Doppler measurements of the intra-ovarian blood flow were not related to the IVF outcome (data not shown). This is in accordance with the results of Tekay *et al.* (1995), but does not agree with the results of Baber *et al.* (1988) and Weiner *et al.* (1993). In their reports, the latter reported a significant difference in the RI value (Baber *et al.*, 1988) and the PI value (Weiner *et al.*, 1993) between patients who conceived and those who did not. However, the stimulation protocols were different in the two studies.

In a previous report, we studied physiological modifications in the uterine blood flow during stimulation for IVF (Bassil *et al.*, 1995). In this study, we present modifications in the ovarian blood flow determined by means of colour Doppler. It is the first prospective controlled study evaluating ovarian vascularity by RI measurement in IVF. In this study, we used a homogeneous group of patients with the same favourable conditions for Doppler measurement. We demonstrated that the ovarian blood flow is differently affected from patient to patient. However, GnRHa down-regulation increases the ovarian RI and an oestradiol concentration >500 pg/ml is needed to improve ovarian vascularity. The intra-ovarian RI value after GnRHa down-regulation appeared to be linked to that at 6 days after the beginning of HMG administration. This RI value indicated the sensitivity of the patient to GnRHa inhibition. No relationship was found between RI value and pregnancy outcome.

The data obtained in this study suggest that the RI value is a good indicator of ovarian inhibition and might be used to determine the optimal timing for the beginning of HMG administration in patients undergoing IVF stimulation with a long protocol. When the RI is high (>0.56), delaying the start of stimulation may increase the oocyte yield, and require lower doses of HMG. On the other hand, in such women, starting with a higher dose of HMG may improve blood flow much faster, also resulting in a higher number of follicles. Further studies are needed to evaluate the relationship between the RI value and the stimulation outcome.

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