



Comparison of natural and artificial cycles in frozen-thawed embryo transfer: A retrospective analysis of 1696 cycles

Dondurulmuş çözülmüş embriyo transferinde doğal siklus ve yapay siklusun karşılaştırılması: 1696 döngünün retrospektif analizi

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Abstract

Objective: This study aimed to compare the pregnancy outcomes of natural cycles (NC) and artificial cycles (AC) in patients undergoing endometrial preparation for frozen-thawed embryo transfer (FET).

Materials and Methods: This retrospective cohort study was conducted in a private infertility clinic between September 2016 and January 2021 and reviewed 1696 FET cycles. Among these FET cycles, endometrial preparation protocols that are performed as the NC (group 1) and AC (group 2) were analyzed. Outcome measures were live birth rates (LBR), clinical pregnancy rates (CPR), implantation rates (IR), and miscarriage rates (MR).

Results: The mean serum estradiol level before progesterone supplementation was significantly higher in group 2, whereas endometrial thickness before progesterone supplementation was higher in group 1 ($p<0.05$). The mean number of transferred embryos and embryo quality score rates regarding cleavage and blastocyst stages were similar in both groups. The IR and MR were similar between groups ($p>0.05$). Additionally, CPR and LBR were similar in groups 1 (39.2% and 32.8%) and 2 (37.3% and 28.5%) ($p=0.517$, $p=0.134$, respectively). Multivariate logistic regression analyses revealed that female age at embryo freezing time and the number of transferred embryos were predictable variables of live birth [odds ratio (OR): 0.970, confidence interval (CI): 0.948-0.991, $p<0.05$, and OR: 1.359, CI: 1.038-1.780, $p<0.05$, respectively].

Conclusion: Suitable endometrial preparation is essential to obtain successful pregnancy rates; however, no superiority was determined in NC or AC protocols in frozen-thawed cycles. One of these protocols may be performed depending on menstrual regularity and clinical experience.

Keywords: Infertility, assisted reproductive techniques, cryopreservation, embryo transfer, pregnancy outcome

Öz

Amaç: Bu çalışma donmuş çözülmüş embriyo transferi için endometriyal hazırlık yapılan hastalarda doğal ve yapay siklusların gebelik sonuçlarını karşılaştırmayı amaçlamaktadır.

Gereç ve Yöntemler: Bu retrospektif kohort çalışması Eylül 2016 ile Aralık 2020 arasında özel bir infertilite kliniğinde yürütülmüştür. Toplamda 1696 siklus gözden geçirildi. Bu siklulardan, endometriyal hazırlık protokolleri doğal siklus (grup 1) ve yapay siklus (grup 2) olarak gerçekleştirilenler bu çalışmada incelenmiştir. Sonuç ölçütleri canlı doğum oranları, klinik gebelik oranları, implantasyon oranları ve düşük oranlarıydı.

Bulgular: Grup 2'de progesteron takviyesi öncesi ortalama serum estradiol seviyesi anlamlı olarak yüksek iken, progesteron takviyesi öncesi endometriyal kalınlık grup 1'de daha yüksekti ($p<0.05$). Gruplar arasında ortalama transfer edilen embriyo sayısı ve klivaj ve blastosist evreleri ile ilgili embriyo kalite skor oranları benzerdi. İmplantasyon ve düşük oranları gruplar arasında farklı değildi ($p>0,05$). Ayrıca, klinik gebelik ve canlı doğum oranları grup 1

PRECIS: The impact of natural and artificial endometrial preparation protocols is similar regarding pregnancy outcomes in frozen-thawed embryo transfer cycles.

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(%39,2 ve %32,8) ve grup 2 (%37,3 ve %28,5) arasında benzerdi (sırasıyla, $p=0,517$, $p=0,134$). Çok değişkenli lojistik regresyon analizleri, embriyo dondurma zamanındaki kadın yaşının ve transfer edilen embriyo sayısının canlı doğumun predikte edilebilir değişkenleri olduğunu ortaya çıkardı [risk oranı (OR): 0,970, güven aralığı (GA): 0,948-0,991, $p<0,05$ ve OR: 1,359, GA: 1,038-1,780, $p<0,05$, sırasıyla].

Sonuç: Başarılı gebelik oranlarını elde edebilmek için uygun endometriyumun hazırlanması mutlak gerekli olsa da, donmuş çözülmüş sikluslarda doğal veya yapay siklus protokolleri arasında bir üstünlük görülmemektedir. Bu protokollerden biri adet düzenine ve klinik deneyime bağlı olarak uygulanabilir.

Anahtar Kelimeler: Infertilite, yardımcı üreme teknikleri, dondurarak saklama, embriyo transferi, gebelik sonucu

Introduction

Frozen-thawed embryo transfer (FET) has become a promising approach in assisted reproductive technique cycles in recent years due to cryopreservation technique improvements. Survival rates, embryo morphology, and pregnancy outcomes have improved in FET cycles after the widespread use of the vitrification method relative to slow freezing for cryopreservation^(1,2). Frozen-thawed cycles are considered as an option for clinicians to use the freeze-all strategy, prevent ovarian stimulation syndrome, and perform preimplantation genetic testing (PGT) in recent years^(3,4). Moreover, some studies revealed higher pregnancy outcomes in FET cycles than fresh ones⁽⁵⁻⁷⁾. Favorable results have been attributed to better synchronization between the endometrium and transferred embryo in FET cycles^(5,6).

With the increased use of FET cycles and knowledge on the importance of endometrial thickness, maturation, and receptivity in achieving pregnancy, clinicians have more closely focused on optimal endometrial preparation. Numerous protocols are available; however, the most commonly used are natural cycle (NC) [true (tNC) or modified NC (mNC)] and artificial cycle (AC). The tNC occurs by preparing the endometrium for implantation with endogenous hormones in patients with regular menstrual cycles. Luteinizing hormone (LH) surge and ovulation are determined by serial ultrasonographic examination of follicular growth, serum LH level measurement, or urinary LH kits. However, it might be difficult even in the frequent follow-up and monitoring. To overcome this problem, mNC is the preferred protocol in which human chorionic gonadotropin (hCG) is administered to trigger ovulation when the leading follicle is measured at ≥ 18 mm. Embryo transfer (ET) is performed according to the embryonic stage after ovulation is identified in both protocols. In AC protocol, exogenous estrogen (E2) and progesterone are administered to prepare the endometrium and provide endometrial maturation, respectively. It might be used in all patients with or without regular cycles for controlled cycles and optimal ET timing^(3,4).

Despite the growing evidence on the impact of endometrial preparation protocols for the success of FET cycles, any protocol superiority is unclear. Generally, pregnancy outcomes are similar between NC and AC^(4,8-11). However, others have presented that NC has higher pregnancy rates than AC^(3,12,13). Additionally, many modifications are made regarding these protocols, and choosing one of these approaches may be challenging and generally depends on clinicians' preferences. Therefore, this study aimed to compare the impact of the

endometrial preparation protocols on pregnancy outcomes in a large number of cycles in patients undergoing FET with NC and AC.

Materials and Methods

This observational cohort study was retrospectively conducted at a private infertility clinic (Novaart IVF Center) in Ankara from September 2016 to January 2021. Approval was obtained from the local Ethics Committee of Gazi University Faculty of Medicine (approval number: 2021-918, date: 23.03.2021). All FET cycles were reviewed from the medical records of the clinic. All completed frozen-thawed cycles performed in the same clinic were included in the study. A total of 1696 thaw cycles of 1297 patients were evaluated. Some multiple cycles were recruited from the same patients; however, the same endometrial preparation protocol was performed in these patients during each FET cycle. All included patients had normal intrauterine cavities assessed by hysteroscopy or hysterosalpingography, of whom two groups were formed according to endometrial preparation protocol for FET cycles: NC (group 1) and AC (group 2). Patients with previous intrauterine surgery; intrauterine lesions, such as polyps, fibroids, and septum; undergoing PGT cycles; and diagnosed with endometriosis, autoimmune diseases, and antiphospholipid antibody syndrome were excluded from the study.

Endometrial preparation protocols were performed according to clinicians' decisions. NC was performed in patients with regular menstrual cycles, whereas AC in patients who are oligo-ovulatory with a possible unpredictable follicular growth pattern and in some who are normo-ovulatory. In NC, baseline monitoring was started on day 3 of the cycle. Patients were then followed up with 2-3 days intervals by ultrasound and serum E2, LH, and progesterone level measurement to evaluate the endometrial thickness and follicle growth, and determine ovulation timing. LH surge (>15 mIU/mL) was determined with the blood test performed on the morning during follow-up visits. Progesterone supplementation was administered by vaginal gel (Crinone 8% gel, Merck Serono, Turkey) at 90 mg once a day or vaginal micronized progesterone capsules (Progestan, Koçak Farma, Turkey) at 200 mg three times a day for luteal phase support (LPS) 48 h after detecting serum LH surge and 24 h after the leading follicle collapse. Progesterone was used for 3 or 5 full days before cleavage stage or blastocyst stage ET, respectively. Thawing of embryo and transfer was performed one day after 3 or 5 full days of progesterone supplementation. Progesterone was continued until the 10 weeks of gestation.

In AC, oral 2 mg of estradiol (Estrofem, Novo Nordisk, Turkey) two times daily was started on day 3 of the menstrual cycle. Patients were evaluated after 7 days, with 3-4 days intervals by transvaginal ultrasound and serum E2 levels to adjust the estradiol dosage relative to the endometrial thickness and E2 concentration. Both vaginal (Crinone 8% gel, Merck Serono, Turkey) at 90 mg once a day or vaginal micronized progesterone capsules (Progesteron, Koçak Farma, Turkey) at 200 mg three times a day for LPS was administered when the endometrial thickness was ≥ 7 mm in diameter and serum E2 was >150 pg/mL. ET was performed after 4-6 full days of progesterone administration according to the embryonic stage. E2 and progesterone administration were continued until the end of the first trimester of gestation.

The vitrification method was performed for cryopreservation in all FET cycles. All embryos were fertilized by intracytoplasmic sperm injection procedure in the same clinic and obtained from the fresh cycles of the same patients who undergo FET cycles. Thawing of embryos was performed on the planned ET day. The morphology and cell number of embryos were evaluated on the transfer day to determine their quality. Good quality embryos were defined as grades 1 and 2 according to the grading system of Hardarson et al.⁽¹⁴⁾ for day 3 cleavage stage embryos. For day 5 blastocyst stage embryos, good quality embryos were defined as ≥ 3 BB according to the Gardner and Schoolcraft embryo grading system⁽¹⁵⁾. One to two ET was performed under the transabdominal ultrasonographic guidance using a flexible catheter (Wallace; Irvine Scientific, Santa Ana, CA).

The primary outcome measure was live birth rates (LBR). Secondary outcome measures were clinical pregnancy rates (CPR), implantation rates (IR), and miscarriage rates (MR).

Clinical pregnancy was proven when a gestational sac or a fetus was monitored by ultrasonography. The biochemical loss was defined as pregnancy loss before the gestational sac was identified on ultrasonography. Miscarriage was determined when a non-viable fetus before 23 weeks of gestation was delivered. Live birth was defined as the delivery of a viable fetus at ≥ 23 weeks of pregnancy. Implantation rate was determined as the ratio of the total number of the gestational sac to the total number of transferred embryos.

Statistical Analysis

Data were analyzed by Statistical Package for Social Sciences (SPSS, version 21.0, Statistics, 2013, Chicago, IBM, USA). Normality tests, including visual (histograms and probability plots) and analytical methods (Kolmogorov-Smirnov test), were performed to determine the normal distribution of variables before analysis. The Student's t-test was used to compare normally distributed parametric variables between groups. Non-normally distributed metric data were compared by the Mann-Whitney U test if required. The chi-square test or Fischer's exact test was used to compare categorical variables. Data were presented as mean \pm standard deviation (SD), percentages, and median (25-75 percentile). Multivariate logistic regression

analysis was performed to identify independent variables in predicting live birth. Cycle numbers and fresh cycle pregnancy were used as categorical covariates in the multivariate logistic regression analysis. The model fit was evaluated by Hosmer-Lemeshow goodness of fit statistics. Statistical significance was accepted as $p < 0.05$.

Results

This study analyzed 1696 completed FET cycles with two different protocols for endometrial preparation. Group 1 (NC) had 311 cycles and group 2 (AC) had 1385 cycles. Of 1696 cycles, clinical pregnancy was obtained in 638 (37.6%) and live birth was delivered in 497 (29.3%).

Baseline characteristics of groups are presented in Table 1. No significant differences were found except for the causes of infertility ($p < 0.001$) between groups. Among the causes of infertility, no ovulatory dysfunction was determined in group 1, as expected. Unexplained infertility was the frequent etiology in groups 1 and 2 (56.6%, and 46.8%, respectively).

The comparison of the cycle and pregnancy outcomes between groups is shown in Table 2. The mean serum estradiol level one day before progesterone supplementation was significantly higher in group 2 (223.2 ± 74.2) than that in group 1 (215.5 ± 56.0) ($p < 0.05$). However, endometrial thickness before progesterone supplementation was significantly higher in group 1 (10.3 ± 1.4 , respectively) than that in group 2 (10.0 ± 1.5 , respectively) ($p < 0.05$). The mean number of transferred embryos and embryo quality scores regarding both cleavage and blastocyst stages were similar among the two preparation protocols. LBR were similar between groups 1 (32.8%) and 2 (28.5%) ($p = 0.134$). CPR were also similar between the groups (39.2% and 37.3%, respectively) ($p = 0.517$). Besides, IR and MR were not different between groups (24.9%, 23%, $p = 0.355$, and 6.4%, 8.7%, $p = 0.183$, respectively).

Multivariate logistic regression analysis to predict live birth was presented in Table 3. Female age at the time of embryo freezing and the number of transferred embryos were found as independent predictors of live birth [odds ratio (OR): 0.970, confidence interval (CI): 0.948-0.991, $p < 0.05$, and OR: 1.359, CI: 1.038-1.780, $p < 0.05$, respectively].

Discussion

This study revealed that LBR, CPR, IR, and MR were similar among natural and artificial endometrial preparation protocols performed for FET cycles. Our NC group may be accepted as mNC due to progesterone supplementation for LPS. Despite the well-known importance of endometrial preparation for achieving pregnancy, the optimal protocol in patients who undergo FET cycles is still debatable.

Generally, NC (tNC or mNC) and AC are mostly evaluated endometrial preparation protocols for FET cycles in the literature. However, results are inconsistent regarding any of these preferred protocols. A recent Cochrane review reported

Table 1. Comparison of baseline characteristics of the patient groups who received endometrial preparation with natural cycle and artificial cycle before FET

Variables	Natural cycle (group 1) (311 cycles)	Artificial cycle (group 2) (1385 cycles)	p-value
Age at the time of embryo freezing (years)	33.0±4.8	32.5±5.4	0.092
Age at the time of embryo thawing (years)	33.9±4.6	33.3±5.3	0.060
BMI (kg/m ²)	22.8±2.2	23.1±2.0	0.104
Duration of infertility (years)	5.5±3.6	5.3±4.0	0.278
Duration of cryopreservation (years)	1.1±1.0	1.1±1.2	0.253
Number of prior IVF attempts	1 (1-2)	2 (1-3)	0.242
Number of previous thaw cycle attempts	1 (1-2)	1 (1-2)	0.100
Fresh cycle pregnancy (%)	14.1	15.5	0.542
Infertility etiology (%)			<0.001
Unexplained	56.6	46.8	
Male factor	31.5	25.7	
Tubal factor	9	6.9	
Ovulatory dysfunction	-	18.6	
Mixt	2.9	2.1	

Data were presented as mean ± SD, percentage, and median (25-75 percentile). BMI: Body mass index. A p-value of <0.05 was considered significant, IVF: In vitro fertilization, FET: Frozen-thawed embryo transfer, SD: Standard deviation

Table 2. Comparison of cycles and pregnancy outcomes of the patient groups who received endometrial preparation with natural cycle and artificial cycle before FET

Variables	Natural cycle (group 1) (311 cycles)	Artificial cycle (group 2) (1385 cycles)	p-value
Serum E ₂ level, 1 day prior to progesterone supplementation (pg/mL)	215.5±56.0	223.2±74.2	0.041
Serum LH level, 1 day prior to progesterone supplementation (mIU/mL)	18.1±8.6	16.6±7.7	0.062
Serum P level, 1 day prior to progesterone supplementation (ng/mL)	1.7±1.4	1.5±1.3	0.105
Endometrial thickness, 1 day prior to progesterone supplementation (mm)	10.3±1.4	10.0±1.5	0.002
Number of transferred embryos	1.7±0.4	1.8±0.4	0.090
Embryo stage at the day of transfer (%)			0.001
Cleavage	24.8	34.9	
Blastocyst	75.2	65.1	
Quality scores of Cleavage stage embryos (%)			0.665
Grade 1-2	90.9	92.3	
Grade 3	9.1	7.7	
Quality scores of Blastocyst stage embryos (%)			0.412
≥3 BB	92.3	93.8	
<3 BB	7.7	6.2	
Implantation rates (%)	24.9	23	0.355
Biochemical loss rate (%)	5.5	6.9	0.349
Miscarriage rate, n (%)	20 (6.4)	121 (8.7)	0.183
Clinical pregnancy rate, n (%)	122 (39.2)	516 (37.3)	0.517
Live birth rate, n (%)	102 (32.8)	395 (28.5)	0.134

Data were presented as numbers and percentages. E₂: Estradiol; LH: Luteinizing hormone, P: Progesterone. A p-value of <0.05 was considered significant, FET: Frozen-thawed embryo transfer

a low quality of evidence that LBR was comparable between mNC and AC⁽¹¹⁾. Similarly, no significant difference was found in another review regarding pregnancy outcomes among these two groups⁽¹⁶⁾. However, the authors concluded that tNC with either LPS or not has higher CPR than AC without suppression, although LBR was similar between groups. Comparable pregnancy outcomes among mNC and AC without suppression were also reported in a large randomized controlled Antarctica trial⁽¹⁰⁾. Additionally, two recent studies have found similar IR, CPR, and LBR between NC and AC^(4,9). Our study also showed similar findings in terms of IR, CPR, and LBR among NC and AC groups, which was in line with these previous studies. Contrarily, some other studies reported more favorable reproductive outcomes in either NC^(3,12,17,18) or AC^(19,20) than others in FET. Numerous modifications were presented in these studies, such as NC with or without LPS, AC with or without suppression, and different dosages or types of exogenous E2 in AC. Therefore, controversial results are speculated to be attributed to these different endometrial preparation approaches. Moreover, heterogeneous study populations, including patients with only regular menses or both regular and irregular menses, could also contribute to these inconsistent results. Two reviews have reported comparable outcomes in CPR and LBR among tNC and mNC in which hCG has been used to trigger ovulation during NCs^(13,16).

Outcomes of AC and mNC are comparable as in our and other recent studies⁽²¹⁾; however, some advantages or disadvantages are determined in both treatment protocols. The main superiorities of hormonal endometrial preparation include the timing flexibility for an ET in clinics that deal with many cycles and the unnecessary regular menstrual cycles. Additionally, the cycle cancellation due to unexpected follicle development is very rare in artificial endometrial preparation cycles. Likely, no cycle cancellations were determined in our study population. Interestingly, a recent study revealed better outcomes in AC in which spontaneous follicles developed contrary to previous reports⁽²²⁾.

The possible disadvantage of AC is that serum estradiol and progesterone levels are directly correlated with the live birth⁽²³⁾, thus an increased dose of steroid hormones may be required in patients with thin endometrium and having difficulty in reaching the desired serum steroid hormone levels. In these individualized treatment cycles, an increased risk of thromboembolic events related to higher doses of steroid hormones besides other risk factors for thrombosis is possible. This problem can be avoided by using daily low molecular weight heparin injections to reduce the risk of iatrogenic complications. However, this adverse effect is too rare in usual doses of estradiol hemihydrate (2 mg, 3 or 4 times/day) and micronized progesterone (200 mg 3 or 4 times/daily), which is approximately equal to the low dose Ethinyl estradiol-containing oral contraceptive pills. This event has not been previously reported in AC thaw studies, and no case was complicated with thromboembolic events in our study. mNC is a patient-friendly approach in economic burden and unexposed to adverse effects of exogenous E2. However, it is unsuitable for patients with oligo-anovulatory cycles and requires more frequent visits to detect unexpected ovulation leading to cycle cancellations.

The literature has also investigated the impact of serum estradiol levels and endometrial thickness in FET cycles. The increased endometrial thickness (9-14 mm) is considered a positive factor in favorable pregnancy outcomes⁽²⁴⁾; however, higher estradiol levels may not guarantee thicker endometrium and higher pregnancy outcomes⁽⁹⁾. Two studies that compared NC and AC for endometrial preparation in FET revealed a consistently greater endometrial thickness and lower serum estradiol levels with higher pregnancy outcomes in NC^(17,25), which as referred to the appropriate implantation window in lower estradiol levels combined with thick endometrium. Our study revealed similar findings. Our NC group had relatively higher IR and LBR than the AC group but without significant difference. Lower estradiol levels and the greater endometrial thickness could lead to better synchronization between the endometrial and embryonic stage in the NC group.

Table 3. Multivariate logistic regression analyses of variables to predict live birth

Variables	Live birth		
	Odds ratio	95% Confidence interval	p-value
Age at the time of embryo freezing	0.970	0.948-0.991	0.006
Duration of infertility	0.989	0.959-1.020	0.496
Serum E ₂ level, 1 day prior to progesterone supplementation	1.001	1.000-1.003	0.155
Endometrial thickness, 1 day prior to progesterone supplementation	1.034	0.963-1.111	0.356
Fresh cycle pregnancy ^(a)	1.252	0.931-1.684	0.136
Number of transferred embryos	1.359	1.038-1.780	0.026
Number of prior IVF attempts	0.977	0.903-1.057	0.561
Cycle number ^(a)			0.709

Variable with Superscript ^(a) was selected as the categorical covariate, IVF: In vitro fertilization

The need for LPS in the natural ET cycles is also controversial in the literature. Higher LBR^(26,27), as well as lower MR⁽²⁶⁾, was observed in some studies; however, other studies reported similar pregnancy outcomes with or without LPS in the NC of FET^(28,29). We used progesterone supplementation in all NC for LPS due to possible luteal phase defects in some ovulatory patients. Additionally, insufficient corpus luteum (CL) is suggested to be associated with lower implantation and some pregnancy complications in AC⁽³⁾. Generally, MR was reported higher in AC than NC^(3,8). Our study revealed a relatively higher MR in the AC group, which is explained by the insufficient uterine milieu in AC compared to NC due to insufficient other hormones released from the CL, although exogenous E2 and progesterone were extensively used in AC until the end of the first trimester of pregnancy. Additionally, using progesterone in our NC group may have supported the effectiveness of the CL and decreased the possible pregnancy losses.

The present study revealed that female age at embryo freezing time and the number of transferred embryos were significant variables in predicting live birth. Maternal age is known to be a major determinant factor on pregnancy success in IVF and FET cycles, and the chance of pregnancy notably decreased after 35 years of age⁽¹⁷⁾. Considering the increasing frequency of ovulatory dysfunction with advancing age, cryopreserved embryos should be transferred with a higher number in older patients, especially in AC.

Study Limitations

The main strength of the current study is the comparison of the most commonly used endometrial preparation protocols in a large cohort of patients who undergo FET cycles, as well as the similarly higher rates of transferred good quality embryos in both NC and AC by experienced clinicians and the use of vitrification method in all embryos in a single-center, which may affect favorable pregnancy outcomes. The major limitations are the retrospective study design and the bias potential of medical records.

Conclusion

The pregnancy outcome of NC and AC seems to be similar in FET. The NC is more convenient for patients with regular menses as there is no need for excessive exogenous hormonal therapy. However, frequent monitoring might be bothersome for some patients. The AC is reasonable, especially for irregular cycles, to control the cycle with less monitoring. It was in line with some reports in the literature; however, the results of our large cohort may provide the flexibility in choosing one of these protocols for clinicians to prepare endometrium in FET cycles. Therefore, one of these approaches might be preferred according to patients' characteristics and clinicians' experience.

Ethics

Ethics Committee Approval: Approval was obtained from the local Ethics Committee of Gazi University Faculty of Medicine (approval number: 2021-918, date: 23.03.2021).

Informed Consent: Retrospective study.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: A.E., Design: A.E., Data Collection or Processing: E.D., İ.G., M.F.C.A., E.Ş., A.D.T., M.E., Analysis or Interpretation: İ.G., Literature Search: E.D., Writing: E.D.

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