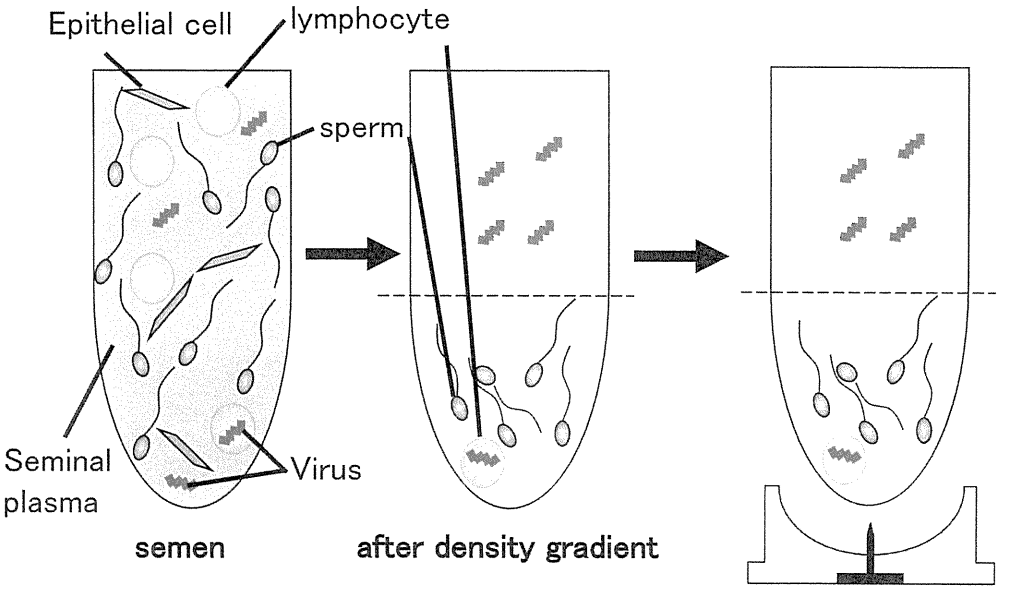
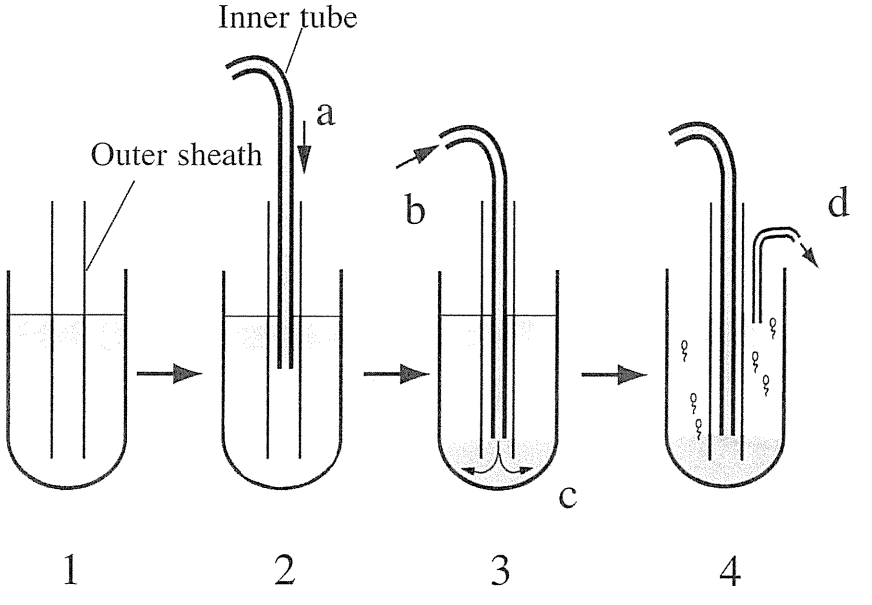
図1. 精液よりHIV-1を除去する方法



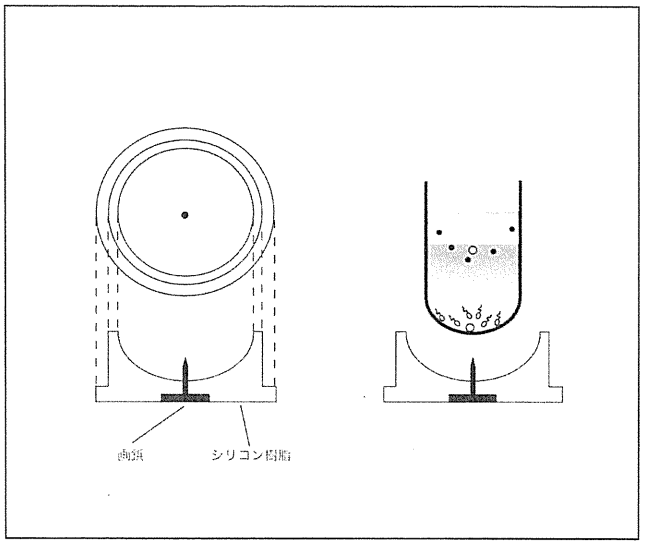
①精液中には精子以外に精漿・白血球・上皮細胞などが含まれ、HIVウイルスは遊離していたり白血球に感染していると考えられている。精液を連続密度勾配にて処理し、遠心分離をかけるることでHIVウイルスとほぼ分離できる。
②上層より吸引して精子を回収すると管壁を伝わり HIV が混入する危険性があるため、試験管底部を穿刺し、 そこより精子を回収する。

図2. swim-up法



①乱流によるウィルス拡散の危険性をさけるため、まずET tubeの外筒を培養液内に入れる。
②外筒の内部にET tubeを挿入し(a)、培養液下層に密度勾配遠心分離された精子を注入する(b,c)。
これにより、ET tubeと培養液は直接接触することなく、精子を注入できる。
③45分後に、swimupした精子は培養液表面より回収される(d)。

図3. 試験管穿孔器



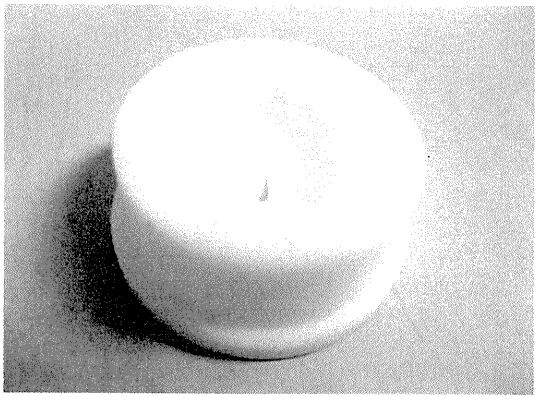
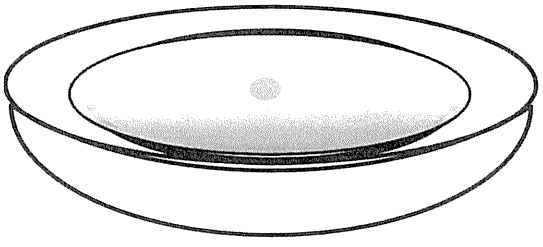


図4.　胚培養液



24時間以上胚培養し交換する際、
Nested PCR法にて胚培養液中の
HIVの有無を確認する。

胚培養液がHIV陰性であれば胚移植を行い、
陽性であれば遺伝子配列解析を行う。

表1. 精液洗浄による精子回収率

Couples(n)	81
Female age(y)	35.9±4.6
IVF cycles(n)	234
ET(n)	225
The sperm cases(n)	165
The sperm collection success cases(n)	163
The sperm collection success rate(%)	98.8
Semen containing(/ml)	$4803 \pm 2423 \times 10^4$
Motility(%)	49±21
Semen containing after swim-up(/ml)	$425 \pm 508 \times 10^4$

表2. 回収精子・胚培養液のHIV陽性数

1)回収精子

	Total number	Positive	Rate
The collection sperm cases	163	5	3%
The collection sperm specimens	334	7	2%

2)胚培養液

	Total number	Positive	Rate
The embryo culture medium cases	233	8	3%
The embryo culture medium specimens	430	10	2%

表3. HIV陽性精子浮遊液の遺伝子配列解析

回収精液163症例中、5症例にPCR法にてHIV陽性を認めた。

①増幅PCR産物の遺伝子配列解析を行った3症例

症例1　夫血液由来HIVと遺伝子配列が一致
症例2　夫血液由来HIVと遺伝子配列が一致
症例3　positive-controlと遺伝子配列が一致

→夫HIV由来と考えられた症例は2症例。

②2症例では増幅PCR産物の遺伝子配列解析を行わなかった

表4. HIV陽性胚培養液の遺伝子配列解析

洗浄精液のHIV陰性を確認後、81例の夫婦に234周期の顕微授精を行った。
胚培養液解析233例中、8例にPCR法にてHIV陽性例が認められた。

①増幅PCR産物の遺伝子配列解析を行った5症例

症例1　夫血液由来HIVと遺伝子配列が一致
症例2　positive-controlと遺伝子配列が一致
症例3　positive-controlと遺伝子配列が一致
症例4　positive-controlと遺伝子配列が一致
症例5　positive-controlと遺伝子配列が一致

→　遺伝子配列解析結果では夫HIV配列と1症例一致した。

②3症例では増幅PCR産物の遺伝子配列解析を行わなかった

表5. 慶応義塾大学病院での HIV-1 discordant couplesにおける治療成績

	N	
HCG positive cases(n)	73	73/225(32.4%)
Clinical pregnancy cases(n)	62	62/225(27.5%)
Live birth(n)	54	54/225(24.0%)
Singleton(n)	47	47/54(87.0%)
Twins(n)	7	7/54(13.0%)
Abortion（n）	17	17/73(23.3%)
Ectopic pregnancy(n)	2	2/73(2.7%)
Mean birthweight of singleton(g)	3269±597	
Mean birthweight of twins(g)	2224±326	

全ての妊娠分娩例で母体・児への感染例は1例も認められなかった。
1例のG6PDdeficiencyが認められた他は、児の異常を認めていない。

表6. 採卵・新鮮胚移植（2009.4～2012.3）

年度	採卵	胚移植	妊娠（%）*	分娩（%）	着床率**
Total	129	57	13（23）	8（14）	17.5
2009	31	31	6（19）	3（10）	15%
2010	57	17	7（41）	5（29）	29%
2011	41	9	0	0	0

＊ 胚移植数を分母とした
** 移植胚総数を分母とした

表7. 凍結胚移植(2009.4～2012.3)

年度	胚移植	妊娠(%)*	分娩(%)	着床率**
Total	102	33(32)	26(25)	23%
2009	37	11(30)	8(22)	20%
2010	30	10(33)	8(27)	22%
2011	35	12(34)	10(29)***	22%

* 胚移植数を分母とした
** 移植胚総数を分母とした
*** 12週を超えるon-going妊娠2例を含む

厚生労働省科学研究費補助金（エイズ対策研究事業）
分担研究報告書

中空糸膜ウイルス除去カラムによる、より効率的な精液中 HIV 除去方法の開発

研究分担者：八幡哲郎、宇都宮徳馬

研究要旨：新たに開発した中空糸膜ウイルス除去カラムを使用して、精液中の HIV をより簡便かつ効率的に除去を行う方法を開発する。本方法による HIV 除去は、従来の swim up 法による HIV 除去の問題点であった精子数の減少を最小限に抑えて運動精子回収率を高めることにより、身体的・経済的な負担の少ない人工授精への応用への可能性も高まる。

A. 研究目的

平成 21 年度の研究によりカラム容積、中空糸本数、膜面積などを変更し数種類の試作カラムの作成に成功し、洗浄後の精子回収率および HIV 除去効率に関し、至適カラムの作成に成功した。平成 22 年度は、引き続き中空糸膜が精子生存率および運動率与える影響に関する検討を実施するとともに、至適カラムを使用して、HIV 感染男性から得られた精液を使用し、HIV 除去に関する有効性の確認を行うことを目的とした。

B. 研究方法

平成 21 年度に作成したカラムのうち、洗浄後の精子回収率および HIV 除去高率が最も良好であった B60 カラム（カラム容量 10ml、中空糸 60 本、中空糸表面積 89-97m2）を使用し、健常男性精液に HIV RNA を混じた感染モデル精液の精子回収率および HIV 除去効率について引き続き検討を行った。また、未治療の HIV 感染男性から得られた精液を使用し、同様に HIV 除去効率を検討した。HIV 感染男性からの精液の提供については、新潟大学伊医学部遺伝子倫理審査委員会での承認を得て、提供男性への説明と同意得て研究を行った。

C. 研究結果

B60 カラムを使用した精子回収率および HIV 除去効率に対する追加検討では、精子回収率は、10 回のカラム洗浄までほぼ 100%の回収が得られ、以降は回収率が

低下し20回洗浄ではおよそ10%まで低下した。Nested PCRによるHIV-RNAの除去効率に関する検討では、3回の洗浄まではHIV-RNAが認められていたが、6回以上の洗浄ではHIV-RNAは認められなかった。8名の未治療のHIV感染男性の精液を用いたHIV除去効率に対する検討でも、感染モデル精液を使用した場合と同様に6回以上の洗浄ではHIV RNAは認められなかった。

D. 考察

現在、HIV感染男性/非感染女性に対する夫婦間および母子間のHIV感染を防ぐための妊娠に対して、改良型Percoll-swim up法による精液中のHIV除去法による医学的介入を行っている。これまでの研究において、本方法により確実にHIVは除去されるものの精子数の減少が大きく人工授精の応用には困難な状況であった。新たに開発した中空糸膜カラムにより、精子回収率を高めつつ、より簡便・効率的に精液からHIVを分離することが可能であった。B60カラムによる6-10回の洗浄が至適条件であった。

E. 結論

新たに開発した中空糸カラムにより、精子回収率を高めつつ、より簡便・効率的に精液からHIVを分離することが可能となり、人工授精への応用が期待できある。

G. 研究発表

学会発表

第28回日本産婦人科感染症研究会（2010/6/5）

新たに開発した中空糸膜カラムによる精液中のHIV除去に関する研究

南川高廣、全錦華、加嶋克則、八幡哲郎、高桑好一、田中憲一、加藤真吾

第23回日本性感染症学術大会（2010/12/11-12）

新たに開発した中空糸膜カラムによる精液中のHIV除去に関する研究

南川高廣、全錦華、加嶋克則、八幡哲郎、高桑好一、田中憲一、加藤真吾

H. 知的財産権の出願・登録状況

なし

厚生労働科学研究費補助金（エイズ対策事業）
分担研究報告書

分担研究者　宇都宮　龍馬
旭化成クラレメディカル株式会社　アフェレシス事業部　学術部

研究要旨
中空糸血漿分離膜を用いた精液中からのエイズウイルス分離研究を広く告知するためにホームページからの情報発信を行うとともに、研究に用いる中空糸血漿分離膜の提供を行った。

A. 研究目的
精液からの HIV 除去を簡便に実施でき、受精の確率を高めることが予想される HIV 除去法の確立

B. 研究方法
旭化成クラレメディカルが提供する中空糸血漿分離膜に精液懸濁液を通過させることにより HIV のみが除去される。この操作を繰り返すことにより受精可能な精液が精製される。

C. 研究結果
研究の内容をホームページにまとめる作業を行うとともに中空糸血漿分離膜 10 本の提供を行った。

D. 考察
HIV が混入している可能性のある、精液中白血球の除去法として、本処置の前に、白血球除去能力のある不織布に浸す対応が考えられた。

E. 結論
ホームページでの情報公開を行い、中空糸血漿分離膜の提供を行った。

F. 健康危険情報
なし

G. 研究発表
1．論文発表
なし
2．学会発表
なし

H. 知的財産権の出願・登録状況
（予定を含む）
1．特許取得
なし
2．実用新案登録
なし
3．その他
なし

以上

研究成果の刊行に関する一覧表

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Original Article

Studies of Assisted Reproduction Techniques (ART) for HIV-1-Discordant Couples Using Washed Sperm and the Nested PCR Method: a Comparison of the Pregnancy Rates in HIV-1-Discordant Couples and Control Couples

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Vol. 62, No. 3, P. 173-176, May, 2009

Jpn. J. Infect. Dis., 62, 173-176, 2009

Original Article

Studies of Assisted Reproduction Techniques (ART) for HIV-1-Discordant Couples Using Washed Sperm and the Nested PCR Method: a Comparison of the Pregnancy Rates in HIV-1-Discordant Couples and Control Couples

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SUMMARY: In this study, the efficacy and safety of assisted reproduction techniques with the sperm-washing method and nested PCR assay were evaluated in HIV-1-discordant couples, as many HIV-1-positive people of reproductive age are getting married and wish to have children safely. Twenty-seven HIV-1-discordant couples (husband, positive; wife, negative) were enrolled in this study. The spermatozoa were separated from semen samples by density gradient centrifugation and the swim-up method. HIV-1 RNA and proviral DNA were checked using nested PCR with a detection limit of one copy before fertilization and before embryo transfer. Clinical outcomes were compared with those of matched control couples. Thirty-eight cycles of in vitro fertilization or intracytoplasmic sperm injection were performed in HIV-1-discordant couples, where the pregnancy rates per embryo transfer and per couple were 60.6 and 63.0%, respectively. These rates were significantly higher than those in control couples ($P < 0.05$). Furthermore, all of the females and babies remained HIV-1 negative throughout the study period. Our data strongly suggest that this technique will allow HIV-1-discordant couples to conceive more safely and effectively.

INTRODUCTION

Recently, people infected with human immunodeficiency virus-1 (HIV-1) have been living longer, since the application of highly active antiretroviral therapy (HAART) has greatly improved survival. Many HIV-1-positive people of reproductive age are getting married and wish to have children safely. It would be possible for an HIV-1-infected male to father children without the risk of HIV-1 transmission if HIV-1-free spermatozoa could be obtained from his semen. The clinical value of sperm washing as well as its risks was first reported in 1992 by Semprini et al. (1), and since then, it has been confirmed by many authors examining both methodological issues and clinical data. In this study, we applied the assisted reproduction technique (ART) for HIV-1-discordant couples in which the man was HIV-positive and the woman was negative, using the swim-up method and nested polymerase chain reaction (PCR) assay and tried to elucidate the efficacy and safety of the procedure.

MATERIALS AND METHODS

Patient couples: First, the patients consulted the Department of Hematology of Ogikubo Hospital, and the HIV-1 infection status of the husband was assessed. At this time, couples were also informed of the details of this treatment by one of the doctors in the study group. After the couples confirmed their desire to undergo the treatment, they were referred to Niigata University Hospital and then were again informed of the details of the treatment by another doctor in the study group with a counselor. The explanation included details of the procedure for ovulation induction, oocyte retrieval, and embryo transfer as well as the risks of these procedures, followed by an explanation of the protocol for confirming the elimination of HIV-1 from the husband's semen. The risk of secondary HIV-1 infection to both mother and baby, if the wife were to conceive, was also thoroughly explained. After the patients confirmed the final decision to participate in this study and gave written informed consent to treatment, the treatment was started. The approval of the ethical committee of Niigata University School of Medicine was obtained.

Semen pretreatments: Semen samples were obtained by masturbation and then tested for sperm concentrations, motility, and deformity. An improved swim-up method was used to collect HIV-1-free spermatozoa from the semen of HIV-1-positive males. Diluted semen was layered over a Percoll solution with a continuous density gradient of 30 - 98% and then centrifuged. We collected the sperm fraction from the end of the tube, and the spermatozoa were collected using the swim-up method as previously described (2). The sperm suspension was divided into two portions, and one half was provided for HIV-1 assessment while the other was cryopreserved in a liquid nitrogen container.

Detection of HIV-1 RNA and proviral DNA: The HIV-1 RNA and proviral DNA were measured by the nested PCR

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method as previously described (2), with a detection limit of one copy. The oocytes obtained from the HIV-1-negative wives were fertilized after confirming that HIV-1 could not be detected in the washed semen samples by the nested PCR method. Furthermore, the fertilized eggs were cultured for 2 or 3 days and were transferred after a negative result was obtained by the nested PCR procedure in the culture medium of the fertilized eggs.

In vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI): The standard long protocol was adopted for most ovulation stimulation cycles. The short protocol was used for patients who were poor responders. If HIV-1 testing for virion RNA and proviral DNA was negative, the other portion of the sperm sample was thawed for use in conventional IVF or ICSI. IVF or ICSI was offered according to the semen profile of each male. The embryo transfer was conducted only when HIV-1 RNA and proviral DNA were negative by the nested PCR assay.

Seroconversion tests: All female partners who underwent ART, even those who did not conceive successfully, were tested for HIV antibodies, HIV-1 RNA, and proviral DNA in the blood 1, 2, and 3 months after the embryo transfer. Furthermore, babies born to the mothers were tested for HIV-1 RNA and proviral DNA at birth or later.

Control couples: Control couples matched to the woman's age who underwent conventional IVF or ICSI in Niigata University Hospital between January 2001 and July 2007 were randomly selected to assess clinical efficacy. During this period, 417 patients aged between 24 and 48 years underwent 814 cycles of IVF or ICSI by the long or short protocol. Within these control couples, 465 cycles of IVF from 261 patients (403 embryo transfers) and 209 cycles of ICSI (182 embryo transfers) from 118 patients were used to compare clinical results to those of HIV-1-discordant couples undergoing embryo transfer.

Statistical analyses: The Student's t test and chi-square test were used to test differences between HIV-1-discordant couples and control couples. Significance was defined as $P < 0.05$.

RESULTS

Twenty-seven discordant couples in which the man was HIV-1 positive and the woman was negative were enrolled in this study at Niigata University Hospital between January 2001 and July 2007. The age of the women ranged from 21 to 41, with a mean of 32.3 years. Of the 27 males, the plasma HIV-1 viral load was <50 copies/ml in 15 patients, and the median plasma HIV-1 viral load of the other 12 was 967 copies/ml (range, 100 - 100,000). In addition, the median CD4 cell count was 377 cells/ml (range, 96 - 700) in 27 patients.

Twenty-seven women underwent ovulation induction 38 times. Of these 27 patients, 5 underwent ovulation induction twice, and another 2 underwent ovulation induction 3 and 5 times, respectively. The remaining 20 patients each underwent induction once. Two cycles were cancelled due to poor response. HIV-1 RNA and proviral DNA were not detected by the nested-PCR assay in any of the 36 of spermatozoa samples collected from 27 patients. HIV-1-negative sperm were used for IVF in 12 couples and for ICSI in 18 couples. To date, fertilized eggs were obtained in 26 women and embryo transfer was performed in all 26 women after confirming that HIV-1 RNA and proviral DNA could not be detected in the culture medium of the fertilized eggs. Three cycles were canceled due to the lack of fertilization.

The clinical pregnancy rate per embryo transfer was 60.6% (Table 1). Of the 27 HIV-1-discordant couples, 17 patients (63.0%) conceived and 22 babies were born. Three cases resulted in early abortion. The multiple pregnancy rate was 25.0%, with 4 sets of twins and 1 set of triplets. HIV-1 RNA and proviral DNA were negative in all of the females and infants throughout the study period. The median observation period of born babies was 58 months (range, 10 - 86). The clinical pregnancy rates per embryo transfer and per couple in the control couples were 30.8% (180 of 585) and 42.5% (161 of 379), respectively. Therefore, the clinical pregnancy rate per embryo transfer as well as that per couple in HIV-discordant couples was significantly higher compared with that in control couples ($P < 0.001$ and $P < 0.05$ by chi-square test, respectively).

Thirteen cycles of IVF were performed in 12 couples, with the clinical pregnancy rates per cycle and per couple being 72.7 and 66.7%, respectively, with a multiple pregnancy rate of 37.5% (Table 2). There were 23 cycles in 18 couples treated by ICSI, with the clinical pregnancy rates per cycle and per couple being 54.5 and 55.6%, respectively, with a multiple pregnancy rate of 16.7% (Table 3). Although the clinical pregnancy rate was higher in the IVF couples, the difference was not significant.

In the control patients, the clinical pregnancy rates per

Table 1. Clinical outcomes of IVF/ICSI cycles in 27 couples

	Total	Range
Couples (n)	27	
Age (y)	32.3 ± 5.0	21 - 41
Cycles (n)	38	
Total gonadotropin dose (IU)	2,022.4 ± 777.4	1,050 - 4,200
Retrieved oocytes (n)	9.8 ± 6.2	1 - 22
Fertilization rate (%)	50.6 (179/354)	
Transferred embryos (n)	2.2 ± 0.6	1 - 3
Implantation rate (%)	34.8 (24/69)	
Clinical pregnancy rate per embryo transfer (%)	60.6 (20/33)	
Clinical pregnancy rate per couple (%)	63.0 (17/27)	
Delivered pregnancy rate (%)	85.0 (17/20)	
Multiple pregnancy rate (%)	25.0 (5/20)	
Maternal seroconversion (n)	0	
Delivered offspring seroconversion (n)	0	

IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection.

Table 2. Comparison of clinical outcomes between HIV-1-discordant couples and control couples undergoing conventional IVF

	HIV couple	Control couple	*P*-value
Couples (n)	12	261	
Age (y)	31.2 ± 5.0	34.2 ± 3.5	not significant
Cycles (n)	13	465	
Retrieved oocytes (n)	10.8 ± 7.6	6.8 ± 4.8	
Fertilization rate (%)	57.4 (74/129)	71.4 (2,269/3,178)	<0.01
Transferred embryos (n)	2.4 ± 0.5	2.2 ± 0.8	not significant
Implantation rate (%)	42.3 (11/26)	16.0 (140/877)	<0.01
Clinical pregnancy rate per embryo transfer (%)	72.7 (8/11)	30.3 (122/403)	<0.01
Clinical pregnancy rate per couple (%)	66.7 (8/12)	42.1 (110/261)	not significant
Delivered pregnancy rate (%)	100.0 (8/8)	74.6 (91/122)	not significant
Multiple pregnancy rate (%)	37.5 (3/8)	12.3 (15/122)	not significant

Table 3. Comparison of clinical outcomes between HIV-1-discordant couples and control couples undergoing ICSI

	HIV couple	Control couple	*P*-value
Couples (n)	18	118	
Age (y)	33.7 ± 4.5	35.6 ± 3.9	not significant
Cycles (n)	23	209	
Retrieved oocytes (n)	9.7 ± 5.6	7.9 ± 4.9	not significant
Fertilization rate (%)	47.3 (105/222)	49.8 (823/1,653)	not significant
Transferred embryos (n)	2.0 ± 0.7	2.0 ± 1.1	not significant
Implantation rate (%)	28.9 (13/45)	15.6 (64/411)	<0.05
Clinical pregnancy rate per embryo transfer (%)	54.5 (12/22)	31.8 (58/182)	<0.05
Clinical pregnancy rate per couple (%)	55.6 (10/18)	43.2 (51/118)	not significant
Delivered pregnancy rate (%)	75.0 (9/12)	81.2 (47/58)	not significant
Multiple pregnancy rate (%)	16.7 (2/12)	10.3 (6/58)	not significant

embryo transfer of IVF and ICSI were 30.3 and 31.8%, respectively (Tables 2 and 3). The implantation rate and the clinical pregnancy rate per embryo transfer were significantly higher in the HIV-1-discordant couples, especially for IVF treatment (The *P*-values appear in Tables 2 and 3; statistical analyses were performed using the chi-square test).

DISCUSSION

The heterosexual transmission rate of the HIV-1 virus is not very high, but a risk does exist. The rate of male-to-female transmission of HIV-1 in stable heterosexual relationships is estimated to be approximately 1 per 1,000 acts of unprotected intercourse (3). The transmission rates are perhaps greater with advanced stages of the disease, the presence of ulcerative genital infection, a history of previous sexually transmitted disease in the female partner, and the presence of postcoital bleeding. Araneta et al. have reported that the risk of transmission with intrauterine insemination (IUI) using non-washed semen from an infected man is 3.52% (4). For HIV-1-discordant couples with male infection, techniques such as sperm washing would further reduce the risk of transmission. The clinical value of sperm washing and the absence of seroconversions were first reported in 1992 (1). Since then, the method has been confirmed with regard to clinical issues by many authors (5-10). They have reported pregnancy rates per IUI cycle ranging from 15 to 31% (Table 4). However, their method may be suboptimal because it has not been proven to remove HIV-1 RNA completely, and they have not checked proviral DNA in infected cells in the semen. Zhang et al. have reported that HIV-1 may be present as proviral DNA in seminal cells in HIV-1-infected men who have achieved undetectable levels of viral RNA in plasma with HAART (11), and this HIV-1 could be transmitted sexually.

Table 4. Results of ART in HIV-1-discordant couples with infected-male partner

Reference	Couple	Cycle	Pregnancy (%)
IUI treatment			
Semprini et al. (1997)	350	1,000	200 (20.0)
Marina et al. (1998)	63	101	31 (30.7)
Weigel et al. (2001)	47	101	15 (14.9)
Bujan et al. (2004)	56	213	37 (17.4)
Nicopoullos et al. (2004)	105	133	25 (18.8)
Savasi et al. (2007)	581	2,400	456 (19.6)
IVF or ICSI treatment		(embryo transfer)	
Weigel et al. (2001)	19	31	15 (48.4)
Sauer et al. (2002)	34	55	25 (45.5)
Pena et al. (2003)	61	100	35 (35.0)
Ohl et al. (2003)	47	41	20 (48.8)
Chu et al. (2005)	92	146	54 (37.0)
Savasi et al. (2007)	160	278	65 (23.0)
Present study	27	33	22 (60.6)

ART, assisted reproduction technique; IUI, intrauterine insemination.

Although IUI therapy may be simpler and less expensive than IVF or ICSI therapy, IVF or ICSI involves a lower exposure of sperm cells compared to that in IUI, which requires millions of sperm to be placed in the uterine cavity. Another advantage of IVF or ICSI over IUI relates to the increase in pregnancy rates per treatment cycle. IVF or ICSI should decrease the number of attempts needed to establish a success-

ful pregnancy, thus further reducing potential viral exposure from repetitive treatment cycles. Since 1998, several groups have reported the results of IVF or ICSI in HIV-1-discordant couples with an HIV-1-infected male partner (3,7,10,12-16). The pregnancy rates reported in these studies were higher than those obtained using the IUI technique (Table 4). In the present study, the pregnancy rates per embryo transfer of IVF and ICSI were 72.7 and 54.5%, respectively. These data were significantly better than those of the control group for both IVF and ICSI treatment (Tables 2 and 3). Although the reason for the higher success rate in HIV-discordant couples than in control couples remains unclear, it is assumed that the females in the HIV-discordant couples, unlike many of those in the control couples, were endocrinologically normal. Furthermore, all of the females and babies remained HIV-1 negative throughout the study period.

Although the number of patient couples treated in this study was smaller that in previously reported studies, we nonetheless were able to establish the safety of the modality. The HIV-1 RNA and proviral DNA were measured twice, just after adjustment of the semen and just before embryo transfer, by the nested PCR method with a detection limit of one copy, for each patient.

In conclusion, the technology employed in this study is considered to offer promising results for HIV-1-discordant couples, allowing those who wish to conceive to do so more effectively and safely. In future, however, it will be necessary to increase the number of patients examined in order to more fully elucidate the safety and efficacy of this technique.

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A case of neonatal alloimmune thrombocytopenia from human platelet antigen 5b incompatibility

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Abstract

Anti-human platelet-specific antigen (HPA) antibody often causes neonatal alloimmune thrombocytopenia (NAIT). The antibody is produced due to the feto-maternal transfusion of incompatible platelets. In this case study, anti-HPA-5b was detected in the serum of a 30-year-old female patient. Using blood or amniotic fluid, the patient' s HPA-5 phenotype was determined to be a+b-, whereas those of the husband, son and fetus were a+b+. From these findings, we concluded that there was an incompatibility of maternal and fetal HPA. Cordocentesis was performed at 34 weeks of gestation and the fetal platelet count was sufficient for vaginal delivery. A transfusion of HPA-matched platelet was prepared. The baby was delivered by vaginal delivery and there were no physical signs of thrombocytopenia.

Case report

A 30-year-old Japanese woman with gravida 2, para 1, gave birth to a female neonate by vaginal birth at 40 weeks of gestation. Two years before, she gave birth to a male neonate with IUGR and congenital duodenal atresia, whose platelet count at the time of birth was 219 $\times 10^9$/L. His platelet count dropped transiently to 79 $\times 10^9$/L on day 14, but returned to 425 $\times 10^9$/L on day 22 without any treatment (Fig. 1). No intracranial hemorrhage, purpura, or petechiae were noted. During this previous pregnancy, the mother's platelet count was 119 $\times 10^9$/L at 38 weeks of gestation and increased

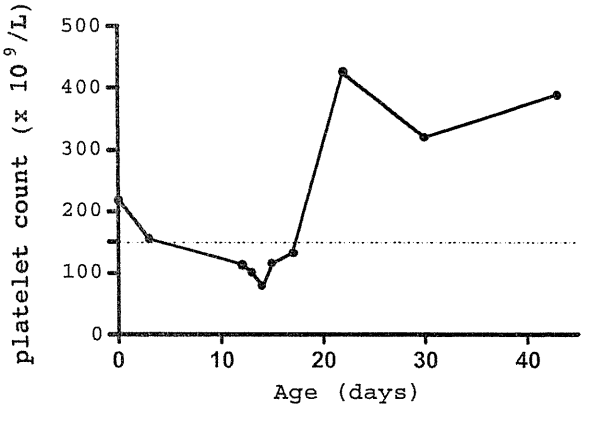

Fig.1 Clinical course of the patient's previous child. Initial platelet count was 219 $\times 10^9$/L. Platelet count at day 14 dropped to 79 $\times 10^9$/L. Without any treatment, platelet count normalized by day 22.

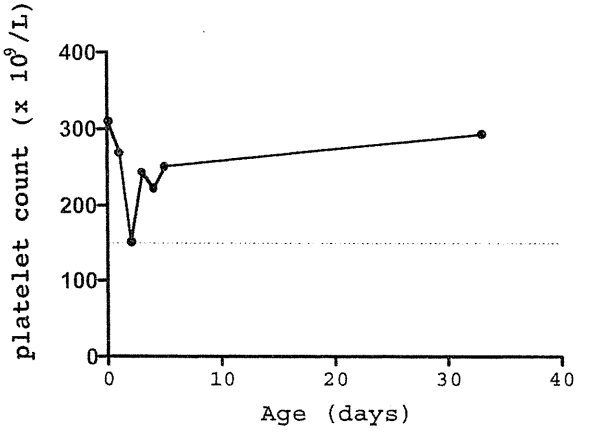

Fig.2 Clinical course of the daughter. Initial platelet count was 310 $\times 10^9$/L. Platelet count at day 2 dropped to 151 $\times 10^9$/L. Without any treatment, platelet count normalized by day 3.

Table 1 HPA type (MPHA method) of the patient, husband,son and fetus

	2b	3a	4a	4b	5a	**5b**	6b
patient	+	+	+	-	+	-	-
husband	+	-	+	-	+	+	-
son	+	+	+	-	+	+	-
fetus	+	+	+	-	+	+	-

The platelet phenotype of the patient was HPA-5(a+b-) while the husband and children platelet phenotypes were HPA-5(a+b+).

to 217 × 10^9/L on day 14 after delivery.

The patient's platelet count was 140 and 141 × 10^9/L at 8 and 14 weeks of gestation, respectively. A platelet-specific antibody, anti-HPA-5b, was detected with a titer of 1:2048 in her serum, using the mixed passive hemagglutination (MPHA) method, suggesting that the former child's thrombocytopenia might have been due to neonatal alloimmune thrombocytopenia (NAIT). Platelet genotyping of the patient, her husband, and her son gave a result of HPA-5 (a+b-), HPA-5 (a+b+) and HPA-5 (a+b+) (Table 1). Because no blood transfusion was given, the patient could have been sensitized during the previous pregnancy through her son; we diagnosed that the son had neonatal alloimmune thrombocytopenia due to the anti-HPA-5b antibody. Since the fetus also had a 50% risk of NAIT, amniocentesis for fetal genotyping was performed at 28 weeks of gestation. The platelet genotyping of the fetus was HPA-5 (a+b+) using amniotic fluid (Table 1). Since the symptoms of NAIT were more severe for the fetus than for the former child, cordocentesis was performed at 34 weeks of gestation to count the fetal platelet and decide the delivery mode. The platelet count was 203 × 10^9/L and hemoglobin was 117 g/L. Peak systolic velocity in the middle cerebral artery of the fetus was measured up to delivery and no evidence of fetal anemia was noted. After vaginal birth, the platelet count of the female neonate was 310 X 10^9/L. The girl had no purpura or petechiae, so HPA-matched platelet transfusion was not attempted. The platelet count of the neonate dropped to 151 × 10^9/L on day 2, but retuned to 243 × 10^9/L on day 3 without any treatment (Fig. 2). Anti-HPA-5b was detected in the umbilical cord blood.

Discussion

The incidence of anti-HPA antibody is 0.6-0.9% in pregnant women [1, 2].

NAIT occurs when the maternal antibodies of an immunized antigen-negative mother cross the placenta and cause destruction of sensitized fetal platelets [3]. NAIT recurs in 70 to 90 percent of subsequent pregnancies, is often severe, and usually develops earlier with each successive pregnancy [4]. Furthermore, severe thrombocytopenia places the baby at risk for intracranial hemorrhage and other bleeding complications [5].

Due to the former birth of an affected child, an occurrence of NAIT was suspected in this case. Using the MPHA method, anti-HPA-5b was strongly detected with a titer of 1:2048 in the patient's serum. Platelet genotyping of the patient, her husband, and her son gave a result of HPA-5 (a+b-), HPA-5 (a+b+), and HPA-5 (a+b+), confirming that the son's disease was NAIT due to the incompatibility of platelet antigen. The patient received no transfusion, so the HPA-5b antigen could have been sensitized by feto-maternal transfusion during the previous pregnancy. Since the fetus had a 50% risk of NAIT because the husband's genotype was heterozygous, an amniocentesis, which is less invasive, was performed for DNA typing at 28 weeks of gestation after informed consent was obtained. The result was HPA-5(a+b+) and it was diagnosed that there was feto-maternal incompatibility of HPA-5b.

For "standard risk" patients, who are defined as women with documented alloimmune thrombocytopenia who did not deliver an infant with an intracranial hemorrhage in a prior pregnancy [6], it is recommended that vaginal delivery be allowed only for patients whose fetuses have a platelet count greater than 100 × 10^9/L