

Table I . The 2009 frequency of four imprinting diseases in Japan in relation to use of assisted reproduction techniques (ART).

Imprinting disorders	Total estimated patient number (95% confidence interval)	The total prevalence of the syndrome	The number of patients after-ART/ Total (%)
BWS	444 (351-538)	1 in 287,000	6/70 (8.6%)
AS	949 (682-1217)	1 in 134,000	2/123 (1.6%)
PWS	2,070 (1,504-2,636)	1 in 62,000	4/261 (1.5%)
SRS	326 (235-416)	1 in 392,000	4/42 (9.5%)

Table I . Abnormal methylation in patients with SRS and BWS.

A.SRS

Case	ART	Abnormal methylation				
SRS-1	IVF-ET	H19 Hypomethylated (mosaic)	PEG1 Hypermethylated	PEG10 Hypermethylated (mosaic)	GRB10 Hypermethylated	ZNF597 Hypomethylated
SRS-2	IVF-ET	H19 Hypomethylated (mosaic)				
SRS-3	IVF-ET	H19 Hypomethylated (mosaic)	PEG1 Hypermethylated (mosaic)			
SRS-4	IVF-ET	H19 Hypomethylated	GRB10 Hypermethylated			
SRS-5	IVF-ET	H19 Hypomethylated (mosaic)	INPP5F Hypermethylated			
SRS-6	-	H19 Hypomethylated				
SRS-7	-	H19 Hypomethylated (mosaic)	ZNF597 Hypermethylated (mosaic)	ZNF331 Hypomethylated (mosaic)		
SRS-8	-	H19 Hypomethylated				
SRS-9	-	H19 Hypomethylated (mosaic)				
SRS-10	-	H19 Hypomethylated				
SRS-11	-	H19 Hypomethylated (mosaic)	PEG1 Hypermethylated			
SRS-12	-	H19 Hypomethylated				
SRS-13	-	H19 Hypomethylated (mosaic)	FAM50B Hypomethylated			
SRS-14	-	H19 Hypomethylated				
SRS-15	-	H19 Hypomethylated				

B.BWS

BWS-1	ICSI	LIT1 Hypomethylated	ZDBF2 Hypermethylated	PEG1 Hypermethylated	NESPAS Hypomethylated (mosaic)
BWS-2	-	LIT1 Hypomethylated			
BWS-3	-	LIT1 Hypomethylated			
BWS-4	-	LIT1 Hypomethylated			
BWS-5	-	LIT1 Hypomethylated			
BWS-6	-	LIT1 Hypomethylated	ZDBF2 Hypomethylated	ZNF331 Hypomethylated (mosaic)	
BWS-7	-	LIT1 Hypomethylated			

Supplementary Table 1. Sequences for PCR primers and conditions used for bisulfite-PCR analysis with a SNP.

Gene	Target	Primer sequence (5'-3')	Amplicon (bp)	Annealing	Accession No.	chromosomal location	
ZDBF2	BS	ZDBF2 BSF1 ZDBF2 BSR1	GT TTT GTT AGT TAG ATT GG AAA ATA AAA ATA AAT TAC CTA AAA ATA AAA AAC	210	57	AC007383	2q33.3
		H19	H19 F2 H19 R1	TAT AT GGT GAT TTT TGG AGG TTT TTT ATA AAT C C C T A T T C C C A A T A A C C C C	220	57	AF125183
SNP	hH19DMR sF1 hH19DMR sR1		AGG TT G G G A G A T G G G A G G A G A T A C G T G G A A T G C C C G A C C T G A A G A T C	395	68		
	BS		GTL2 BSF1 GTL2 BSR1	G G G T T G G G T T T T G T T A G T T G T T T G T A C A A T T T A A C A C A A C T T T C C C C A A A	459	57	
DIRAS3		BS	DIRAS3 BSF1 DIRAS3 BSR1	T G T T G T T T G T T T G A T A T T T G T T G T T C C T T A A A C T T C T A A A C T A A C C C C T C	349	57	AF202543
	SNP		DIRAS3 SF1 DIRAS3 SR1	A C T T A C C T T T C T C G G A G G C A C G A A C A G T T C C T C C C C A A C C T G T A A C	321	65	
		NAP1L5	BS	NAP1L5 BS2F1 NAP1L5 BS2R1	T G A T A G T G G G A A G T T A G T T A A G T G T A A A A A T C T A A A A C C C C T C A A C C A T C	348	
SNP	NAP1L5 SF1 NAP1L5 SR1			G C T G T C A C A G T C C C A C C C T G C C C G C A T C C G C A A G A T C T C T C T G	202	66	
	FAM50B		BS	FAM50B eBSF1 FAM50B eBSR1	G G T T T T G A G G A G A G T T T A G G T T T T A A A A C T C T C T A A A T A A C C A C A C A A C T T A C	334	57
SNP		FAM50B SF1 FAM50B SR1		C A G G T A A T G T T C A C G A G A C G C C A C A G G G G G C T C C T G T T T T C A C G C T G T G	218	67	
		BS	ZAC F ZAC R	G G G G T A G Y G T G T T T A T A G T T T A G T A C R A A C A C C C A A A C A C C T A C C C T A	152	57	AL109755
GRB10	BS		GRB10 BSF2 GRB10 BSR2	G T T A G G G G T T T G Y G Y G T A G A A A A T C C A A T C C C T C R A A A A C T A A	245	55	AC004920
		SNP	GRB10 SF1 GRB10 SR1	G A A C G C G C T A G C A C G A A A A G C C A G T C C C T C G G A G G C T G A G T A T T G	185	69	
	BS		PEG10 BSF1 PEG10 BSR1	T T T A G T T T G G T T A G T T T A G T A T T A G T A T T T A A A A A T A A A A T C C C A C A C C T A A A C	395	55	
PEG1		BS	hPEG1 BSF1 hPEG1 BSR1	A A T T T T A A T T T T G A T G A G T T A T G A G A T A T T T T C A A A T T C A A T A A C A A A C	275	57	AB045582
	SNP		PEG1DMR sF2 PEG1DMR sR2	G T A T C A C G G T G G C G G G A G T C A T G A G C G G A G A C A A T A A G C A A A C	315	61	
		BS	INPP5Fv2 BS4F1 INPP5Fv2 BS4R2	T A G G A A T T T T A A T T A T A A G T T T T G T A A A A T A C A A A C A A C A T T T A A A C C T C	203	55	
BS	LIT1 F LIT1 R		T T T T G G T A G G A T T T T G T T G A G G A G T C C T C A C A C C C A A C A A T A C C T C	307	57	U90095	11p15
	RB1	BS	RB1 BSF2 RB1 BSR1	G T G A A A G T G G G T T T T G G T A G T T T G C T T A A C A T T T C A A A A C T A C C C T A C C	241	59	AL392048
SNP			RB1 SF1 RB1 SR1	C G C C C C C T C T A C G T T T C C T T T T G C C T A G A C G C T G A C C A T T C C C C A A A G	480	68	
		SNRPN	BS	hSNRPN BSF1 hSNRPN BSR1	A G G A G G T T A T G G T A G T G G A T T A G G C A C C A C A A T A A A C A A A C A A A T A A C	383	
SNP	SNRPN-DMR sF3 SNRPN-DMR sR3			A C C G A G G C G A G G A G G C T A T G G A C T G T G C T A C T C C C C C T C T G	335	68	
	ZNF597		BS	ZNF597 BSF1 ZNF597 BSR1	G T T T T T G A T A G G A G T T G T A G A A A G C A A C T A C C A A T A A C T A A A T C C T C	254	57
SNP		ZNF597 SF1 ZNF597 SR1		C G G G T G G G G A A T G C C T T C T T C A A G G A G A A C T T C G A C C A A T C A A A G G C A G G	409	67	
		ZNF331	BS	ZNF331 BSF2 ZNF331 BSR1	G T Y G G G T T T G T T G T T T G T A T A T A T C C C R C C A C C C C T A A A A C C A A C	270	57
SNP	ZNF331 SF2 ZNF331 SR2			C G T G T C A G T G T G C G C G T G T C A G G C T G C G T C A C T G G T C A A A C G	456	69	
	PEG3		BS	PEG3 BS2F3 PEG3 BS2R0	G G T T G T T G A T T G G T T A G T A G A A G T T C T C A C C T C A C C T A A T A C T A C R C A A C	227	55
SNP		PEG3-DMR sF1 PEG3-DMR sR1		C T G T G C C C A C T C T C G G A C T G C A C C T C G G T G C A A A G T C T G G	342	66	
		BS	PSIMCT-1 BSF1 PSIMCT-1 BSR1	G G A T G A G T T G G A T A T A T T T T T T T A C T T A T C A A A C C T A C T A T T T C A A C	328	55	AL110115
NNAT	BS		NNAT BS2F1 NNAT BS2R1	T T T T A G T T A G T G G G T T T T T G T A T T C A T T A C A C C T C C A T C T A C T A C T A A C A C T A T	365	55	AL109614
		SNP	NNAT S2F1 NNAT S2R1	T C A A A C A G C C C A G C G T C A C T T T G A C C T C T T T C C A G C A A G C T G C C T G	237	61	
	BS		L3MBTL BSF0 L3MBTL BSR2	G T G T A G T T T G G A G T G A G G T T T T T T G A A A C C C A A C T C A A A A C T A A A A A A C	331	55	
NESPAS		BS	NESPAS BSF1 NESPAS BSR1	A G T A A A G T T T T T A G G G A G T A G T T G A A C A A A C T A T A A T A A A A C A A A A A A C T A A	312	55	AJ251760
	SNP		NESPAS SF1 NESPAS SR1	C A T G G T A T T T A T C T G T G G G T T C A G G C A G G G T G C T C T C T G T T T A T G	287	62	
		GNAS1A	BS	GNAS1A BSF1 GNAS1A BSR1	G T G T A G T G T A T T T T A T T A T A T G T A A G T A A C A A A A T C T A T T A C C C T C A A A C	236	
SNP	GNAS1A SF1 GNAS1A SR1			G C T G C C T T G C G T G T G A G T G C C G G A T G G C A G G A G T C T G T T T A C C	251	67	
	BS		LINE-1 BSF LINE-1 BSR	T T G A G T T G T G G T G G T T T T A T T T A G T C A T C T C A C T A A A A A T A C C A A A C A	413	50	X58075
Alu		BS	Alu BSF Alu BSR	G A T C T T T T A T A A A A A T A A A A A T A G T G A T C C C A A A C T A A A A T A C A A T A A	152	43	U14568

Supplementary Table II . Results of the first postal survey and the number of four imprinting disorders.

	Total departments	Departments that responded	Response rate (%)	BWS	AS	PWS	SRS
University hospitals	255	131	50.9	59	45	322	46
Institutions for the mentally and physically handicapped	187	83	44.4	11	29	39	3
General hospitals							
≥ 500 beds	227	158	69.6	50	66	213	45
400-499 beds	215	126	58.6	24	39	127	27
300-399 beds	389	225	57.8	29	100	85	8
200-299 beds	348	175	50.3	12	23	63	10
100-199 beds	698	341	48.9	17	73	83	13
≤ 99 beds	839	363	43.2	14	40	60	9
Total	3158	1602	56.3	216	415	992	161

Supplementary Table III . Results of the second postal survey and the number of four imprinting disorders.

	Total departments	Departments that responded	Response rate (%)	BWS	AS	PWS	SRS
University hospitals	40	19	47.5	29	27	75	18
Institutions for the mentally and physically handicapped	27	14	51.9	0	11	11	3
General hospitals							
≥ 500 beds	60	30	50.0	16	22	60	12
400-499 beds	79	47	59.5	4	18	32	2
300-399 beds	56	26	46.4	10	11	24	0
200-299 beds	27	12	44.4	4	8	13	0
100-199 beds	46	23	50.0	5	20	35	4
≤ 99 beds	45	17	43.2	2	6	11	3
Total	380	188	49.5	70	123	261	42

Supplementary Table IV. DNA methylation analyses of the DMRs in the SRS and BWS patients originated from ART.

ASRS																									
SRS	ART	paternally DMRs						maternally DMRs														Non-imprinted			
		ZDBF2	H19	IG-DMR	DIRAS3	NAP1L5	FAM50B	ZAC	GRB10	PEG10	PEG1	INPP5F	LIT1	RB1	SNRPN	ZNF597	ZNF331	PEG3	PSIMCT-1	NNAT	L3MBTL	NESPAS	GNAS	Alu	LINE1
SRS-1	+	45.5	21.0	60.9	45.7	56.5	41.6	44.5	97.1	78.8	99.6	47.7	54.1	49.2	47.8	0.0	58.3	50.3	59.1	51.7	54.0	56.2	53.6	28.3	62.5
SRS-2	+	63.4	25.2	51.5	58.6	46.5	44.0	43.2	58.7	54.7	58.7	53.8	60.1	39.1	49.7	37.7	49.4	51.7	55.8	60.3	43.3	46.9	56.8	24.4	59.4
SRS-3	+	45.7	33.7	52.6	46.6	56.7	54.2	44.9	50.0	49.8	73.8	58.2	43.9	46.7	55.1	44.7	55.8	52.2	43.7	59.0	41.5	49.4	57.8	33.9	46.7
SRS-4	+	42.9	0.3	54.5	56.5	42.9	42.9	53.3	95.9	48.4	53.4	54.6	49.0	47.7	49.5	52.8	56.9	60.2	51.6	57.1	46.2	47.5	59.7	26.6	56.0
SRS-5	+	46.4	26.2	53.6	49.2	44.1	41.3	46.3	55.6	51.0	63.3	97.3	53.2	54.9	45.9	47.7	46.3	52.9	61.1	54.4	40.0	50.6	59.6	22.9	51.6
SRS-6	-	55.2	2.1	51.9	59.4	41.5	48.7	58.8	49.6	48.2	52.7	60.4	48.2	46.2	49.7	41.9	57.7	51.5	59.7	49.4	40.3	39.2	56.8	25.0	70.7
SRS-7	-	43.8	20.3	51.4	39.4	41.4	35.7	53.0	53.8	56.4	52.4	59.8	46.4	62.1	47.7	75.7	18.8	53.5	52.1	46.0	42.6	41.8	59.0	22.9	62.5
SRS-8	-	51.0	3.5	54.9	61.4	55.8	38.8	50.4	56.7	46.4	62.2	44.6	50.7	62.1	45.7	39.3	43.7	51.1	54.3	53.2	32.8	54.7	52.7	24.4	59.6
SRS-9	-	40.2	25.3	49.2	56.2	45.5	39.7	55.7	49.4	44.8	54.7	45.7	42.5	51.0	46.3	46.4	66.9	40.6	61.1	53.6	46.2	39.7	57.4	34.6	59.3
SRS-10	-	41.0	0.4	57.0	49.9	48.4	55.6	45.7	62.3	55.5	52.0	53.9	39.9	37.9	44.5	39.5	55.0	43.1	42.8	55.9	46.9	37.5	55.3	23.9	55.4
SRS-11	-	43.8	16.3	60.6	41.3	40.2	40.3	53.6	55.7	46.0	95.2	56.3	33.9	57.1	46.4	38.7	59.0	60.7	49.8	45.6	43.3	43.4	51.2	27.6	53.3
SRS-12	-	50.5	6.0	54.4	52.4	43.5	48.5	45.3	62.9	44.2	52.1	49.6	37.7	48.2	59.1	51.6	57.8	51.4	62.5	53.8	41.5	49.6	58.2	17.7	51.8
SRS-13	-	51.8	34.1	60.2	46.7	49.3	0.4	53.4	59.1	46.7	40.6	52.9	55.8	47.8	49.7	37.9	56.5	55.1	54.6	51.7	57.3	47.8	55.4	19.4	66.7
SRS-14	-	37.8	2.5	61.5	59.5	39.5	46.9	49.1	55.4	47.4	51.8	55.8	55.8	53.3	43.6	35.0	44.0	51.6	58.7	55.0	47.4	36.6	52.0	26.2	63.2
SRS-15	-	50.5	3.3	53.4	53.4	45.7	49.2	49.6	55.4	45.4	52.0	58.8	53.6	35.5	45.8	41.6	46.4	50.0	59.5	59.3	43.8	36.9	56.3	26.1	64.8
B.WBS																									
BWS-1	+	93.3	57.3	52.0	44.9	47.6	46.6	49.2	45.8	52.2	97.8	42.9	0.0	46.7	46.1	40.3	52.3	46.2	44.0	52.1	44.2	18.3	50.3	17.7	54.8
BWS-2	-	47.3	59.3	49.6	52.2	59.1	48.3	50.0	44.4	51.4	49.8	53.5	0.0	47.7	52.9	41.0	48.1	49.7	59.2	60.0	41.9	43.8	61.6	28.0	73.8
BWS-3	-	47.6	41.9	51.9	47.8	41.2	37.6	41.1	46.0	50.0	53.3	52.7	0.0	42.3	52.7	37.3	52.8	44.1	52.1	51.1	44.4	43.7	58.1	22.2	59.6
BWS-4	-	47.6	61.1	55.0	52.4	54.0	43.6	56.0	52.7	46.0	51.3	52.7	0.7	48.7	52.6	44.4	42.1	45.6	53.6	51.0	45.6	45.0	56.5	26.8	60.5
BWS-5	-	49.5	55.2	47.3	44.0	43.1	45.4	43.2	46.0	44.3	49.8	48.2	0.0	50.8	45.3	50.4	41.3	43.3	51.5	51.9	45.4	44.4	45.7	19.2	57.8
BWS-6	-	95.2	46.3	55.7	42.8	44.7	44.6	46.3	55.4	51.5	48.4	54.8	1.3	46.7	55.0	44.2	16.8	51.5	43.5	50.0	42.0	39.0	54.8	26.3	65.2
BWS-7	-	51.0	51.5	55.8	51.6	46.9	52.7	49.1	58.1	46.3	36.4	51.2	0.0	42.0	45.0	44.7	42.3	55.3	44.4	52.3	58.3	48.8	44.9	27.6	64.6
Normal Blood (n=20)																									
mean±SD		50.8±3.0	48.6±4.2	53.3±1.2	52.6±5.0	45.8±4.1	53.3±5.6	48.4±5.9	53.9±2.3	51.6±2.5	49.8±5.3	52.2±4.1	53.2±3.7	45.9±2.3	49.5±5.1	44.0±8.2	56.6±7.4	49.9±6.1	51.3±3.6	46.3±1.0	41.3±8.4	54.0±6.5	55.8±4.0	24.1±1.3	60.5±3.9
Normal Sperm (n=20)																									
mean±SD		98.2±0.9	96.6±1.0	97.3±0.4	0.4±0.1	0.7±0.8	1.6±0.5	0.5±0.6	0.7±1.0	0.3±0.4	0.4±0.4	0.3±0.2	0.9±0.9	0.6±0.7	0.5±0.6	0.9±0.8	0.7±0.5	0.2±0.4	0.6±0.5	0.2±0.4	0.6±0.7	0.7±0.4	0.8±0.3	21.0±5.0	50.5±2.3

Supplementary Table V. Clinical characterization of patients with SRS and BWS.

A.SRS

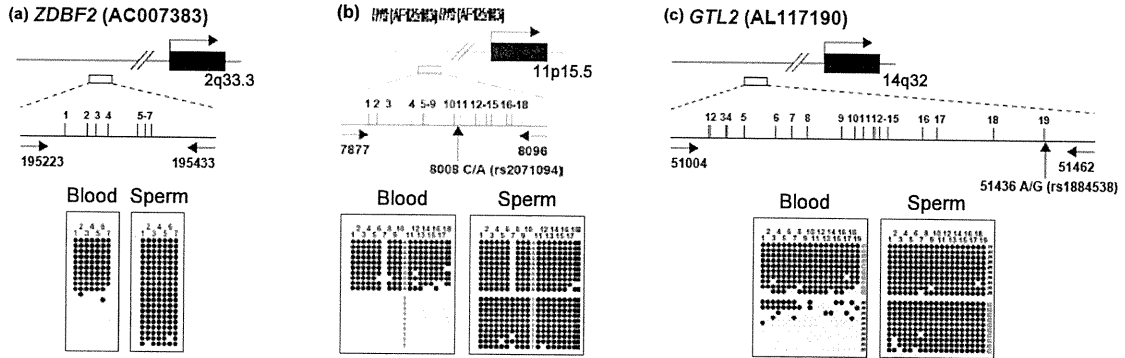
Case	ART	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
SRS-1	+	○	○	—	○	○	—	—	—	○	—	—	○	—	—	—	○	—
SRS-2	+	○	○	—	○	—	—	—	—	○	—	—	○	—	—	—	—	—
SRS-3	+	○	○	—	○	—	—	—	—	○	—	—	○	—	—	—	—	—
SRS-4	+	○	○	—	○	—	—	—	—	○	—	—	—	—	—	—	—	—
SRS-5	+	○	○	—	○	—	—	—	—	—	—	—	○	—	—	—	—	—
SRS-6	—	○	○	—	○	○	—	—	—	○	—	—	○	—	—	—	—	—
SRS-7	—	—	○	—	○	—	—	—	—	○	—	—	○	—	—	○	○	—
SRS-8	—	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
SRS-9	—	○	○	—	○	○	—	—	—	○	—	—	○	—	—	—	—	—
SRS-10	—	○	○	○	○	○	—	—	—	○	—	—	○	○	—	—	○	—
SRS-11	—	—	○	—	○	—	—	—	—	—	—	—	○	—	—	—	—	—
SRS-12	—	○	○	—	○	○	—	—	—	○	—	—	○	—	—	—	○	—
SRS-13	—	○	○	—	○	—	—	—	—	—	—	—	○	—	—	—	—	—
SRS-14	—	○	○	—	○	—	—	—	—	○	—	—	○	—	—	—	—	—
SRS-15	—	○	○	—	○	○	—	—	—	○	—	—	○	—	—	—	—	—

B.BWS

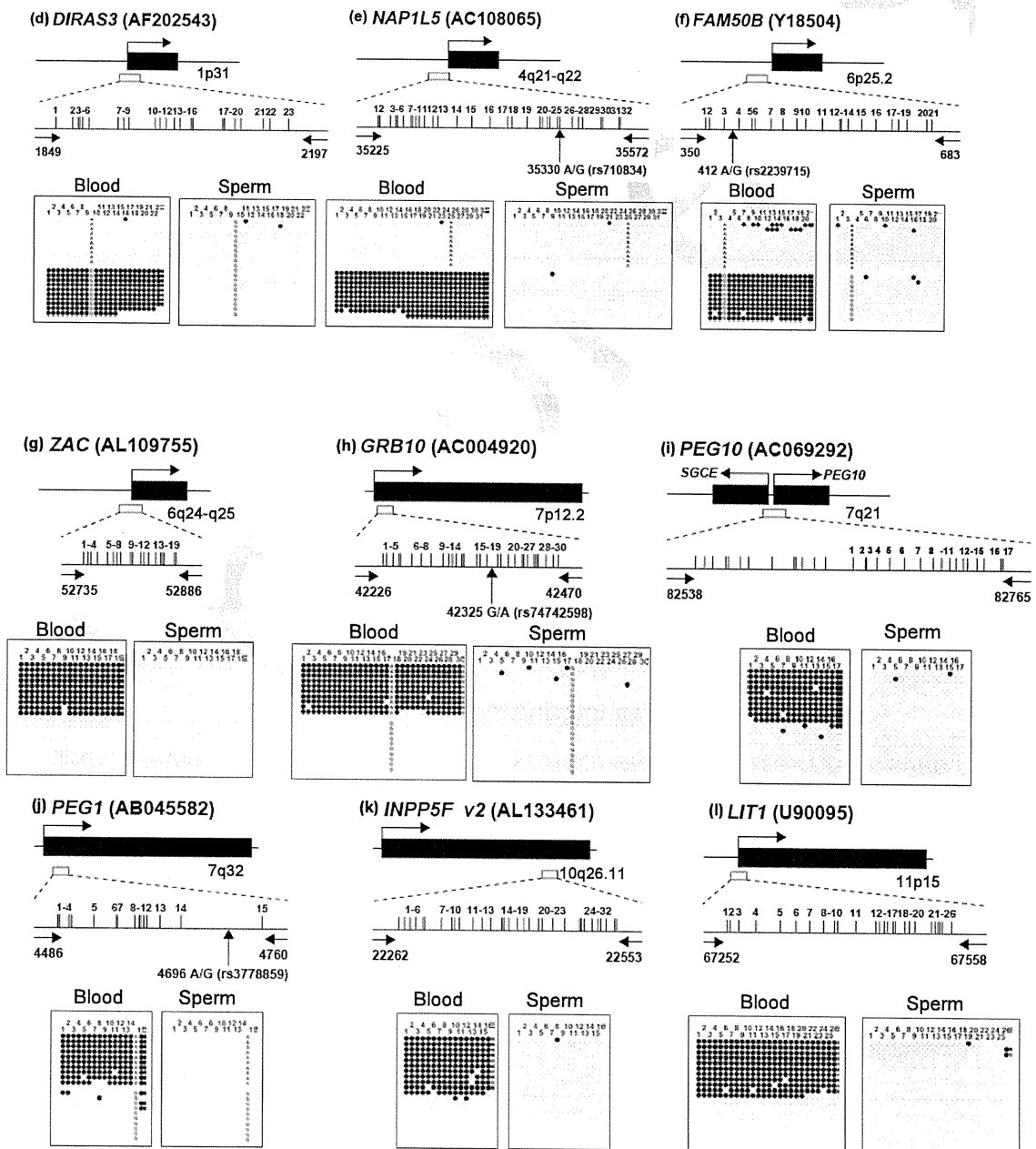
Case	ART	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
BWS-1	+	—	—	—	○	—	—	○	○	—	—	—	—	—	—	—	—	—	—
BWS-2	—	○	—	—	○	—	—	—	○	○	—	—	—	—	—	○	—	—	—
BWS-3	—	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
BWS-4	—	○	—	—	○	—	—	—	—	○	—	—	○	—	—	—	—	—	—
BWS-5	—	—	—	—	—	—	—	—	○	○	—	—	○	—	—	—	—	—	—
BWS-6	—	○	—	—	○	—	—	—	—	○	—	—	—	—	—	—	—	—	—
BWS-7	—	—	—	—	○	—	—	—	—	○	—	—	—	—	—	—	—	—	—

Supplementary Figure 1

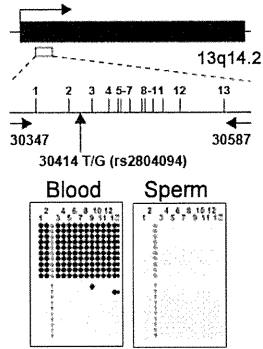
A. Paternal DMRs



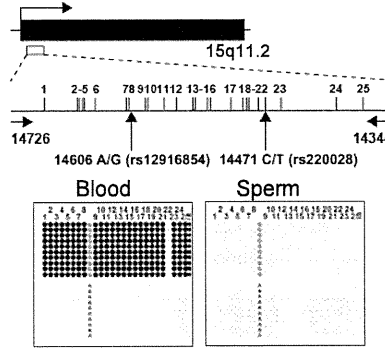
B. Maternal DMRs



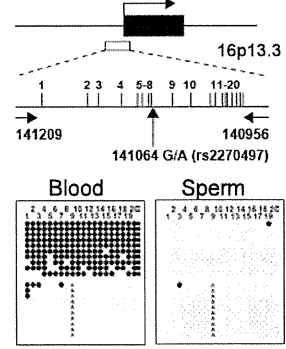
(m) *RB1* (AL392048)



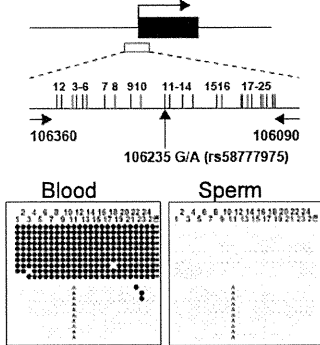
(n) *SNRPN* (U41384)



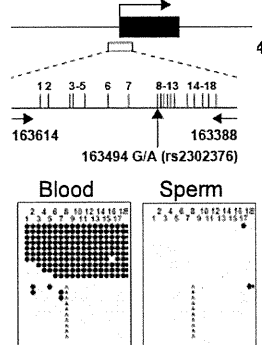
(o) *ZNF597* (AC025283)



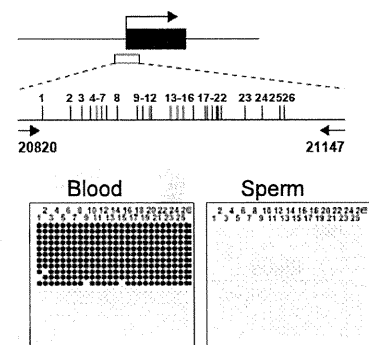
(p) *ZNF331* (AC011487)



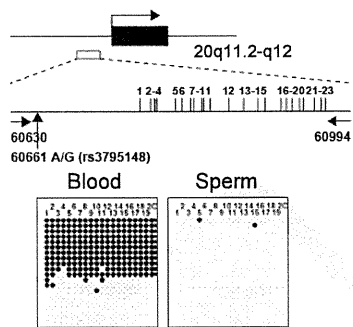
(q) *PEG3* (AC006115)



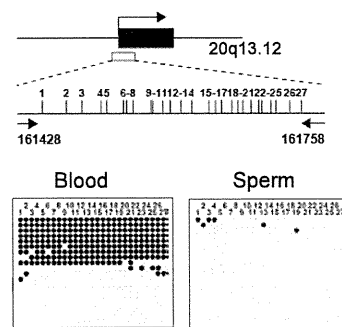
(r) *PSIMCT-1* (AL110115)



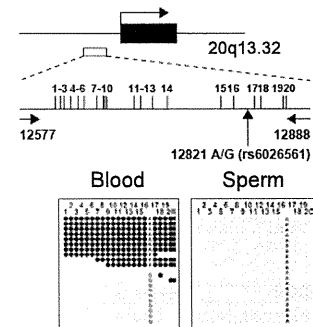
(s) *NNAT* (AL109614)



(t) *L3MBTL* (AL031681)

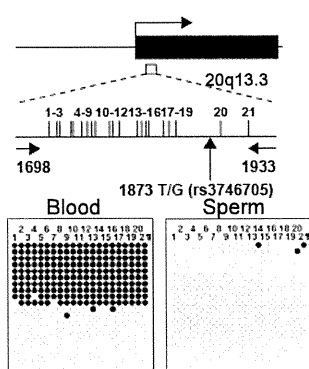


(u) *NESPAS* (AJ251760)

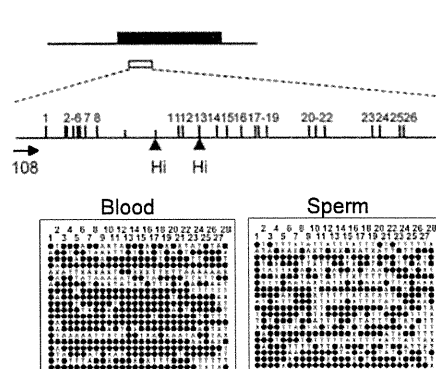


C. Non-imprinted regions

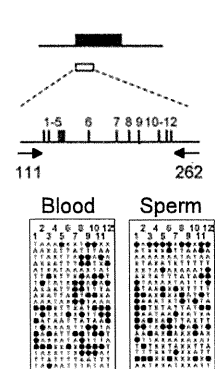
(v) *GNAS1A* (AF246983)



(w) *LINE-1* (X58075)

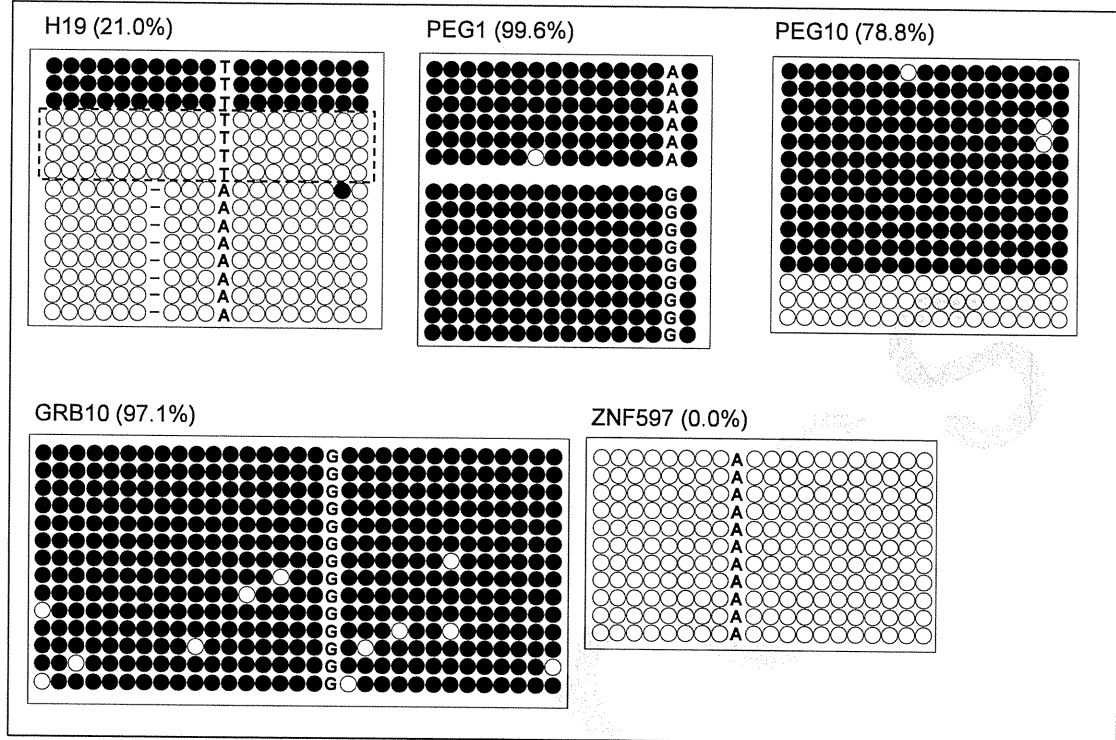


(x) *Alu* (U14568)

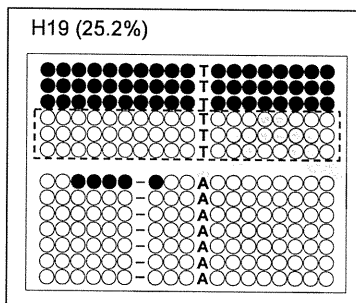


Supplementary Figure 3

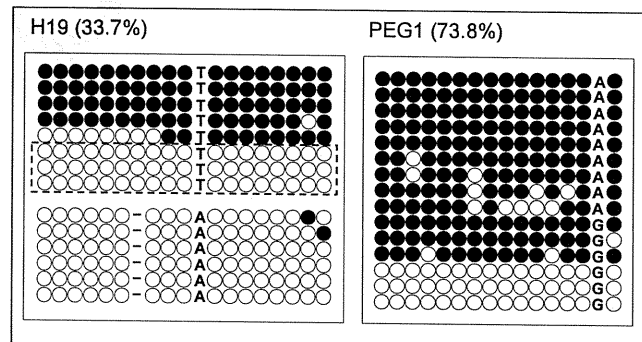
1. SRS-1 (IVF-ET)



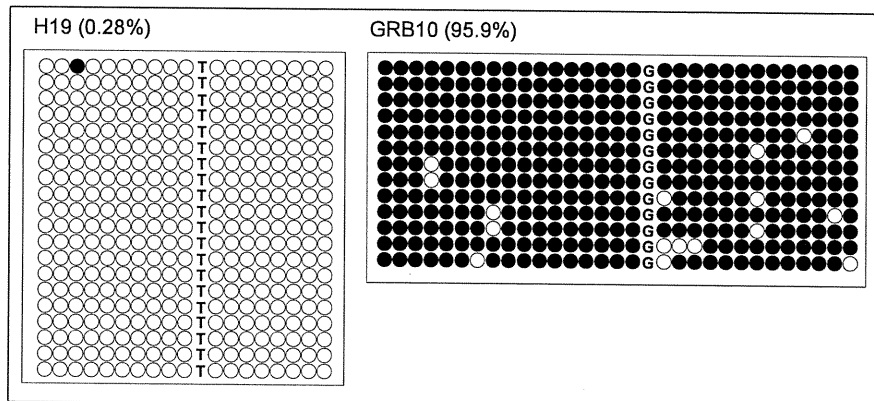
2. SRS-2 (IVF-ET)



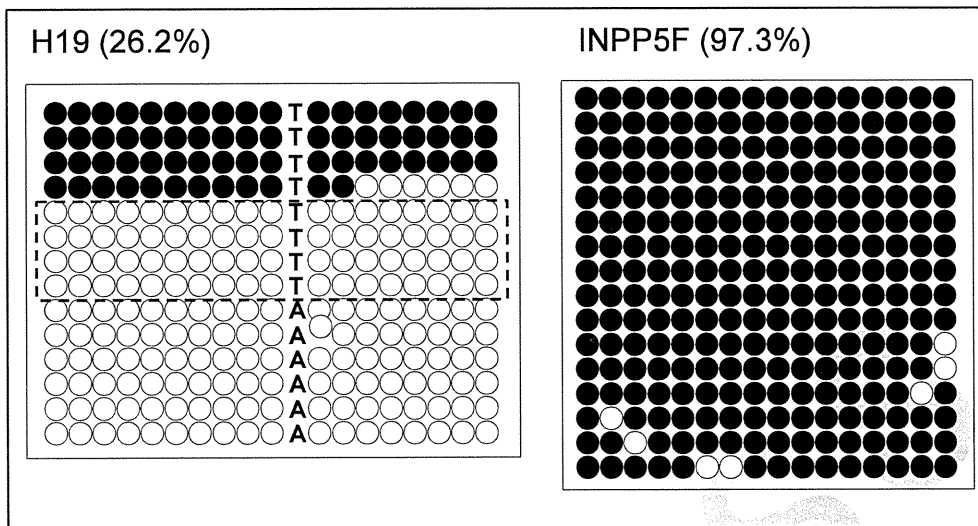
3. SRS-3 (IVF-ET)



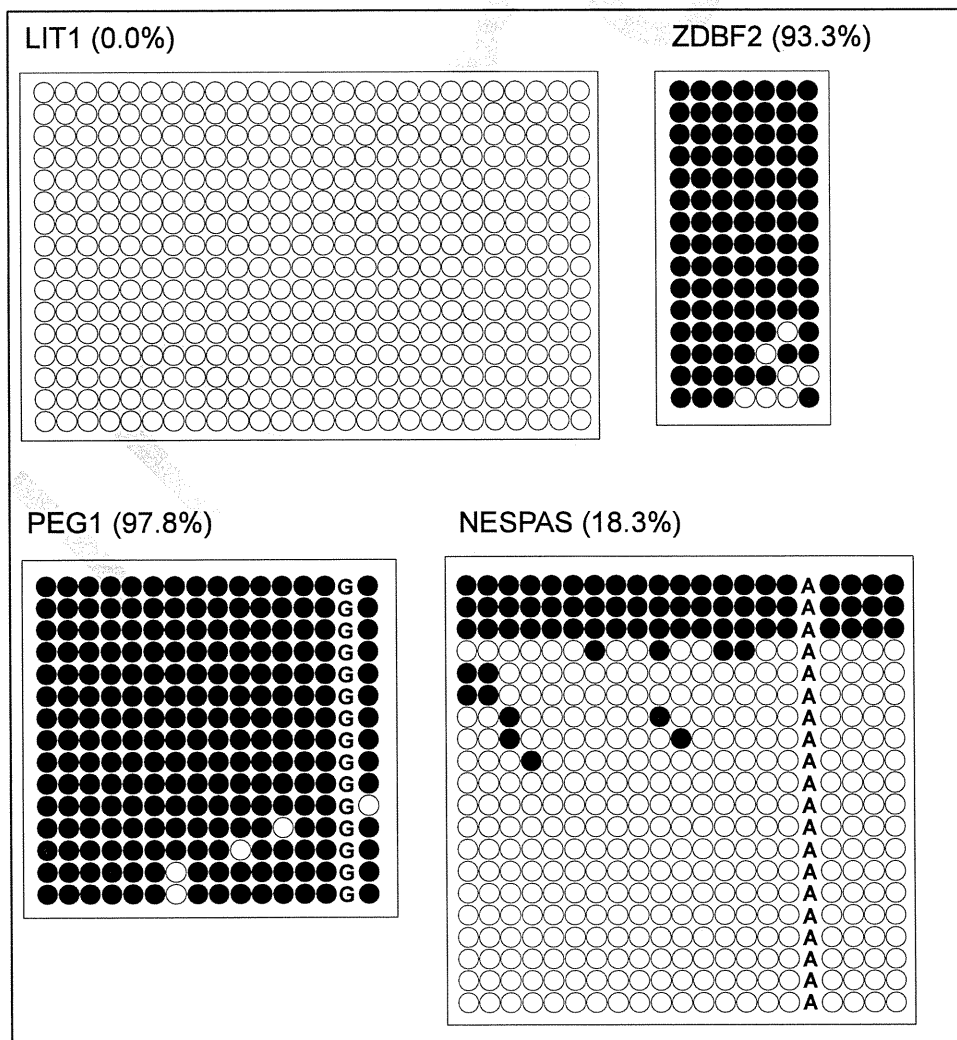
4. SRS-4 (IVF-ET)



5. SRS-5 (IVF-ET)

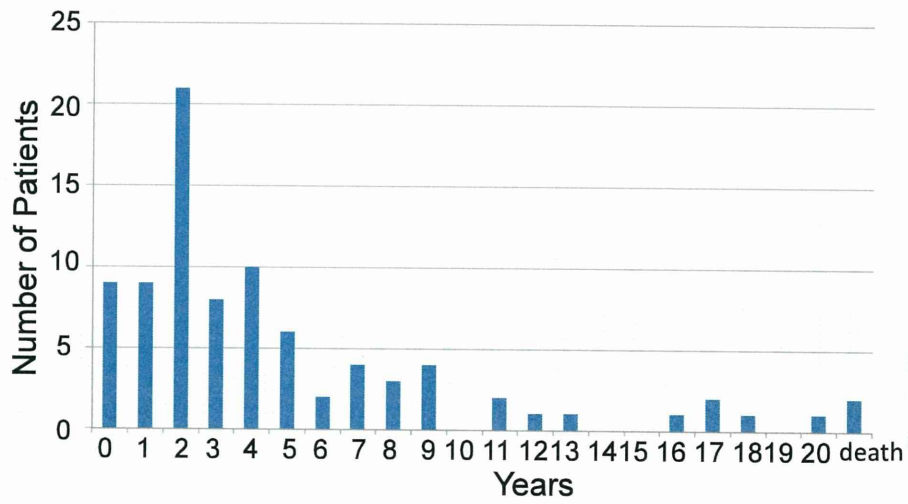


6. BWS-1 (ICSI)

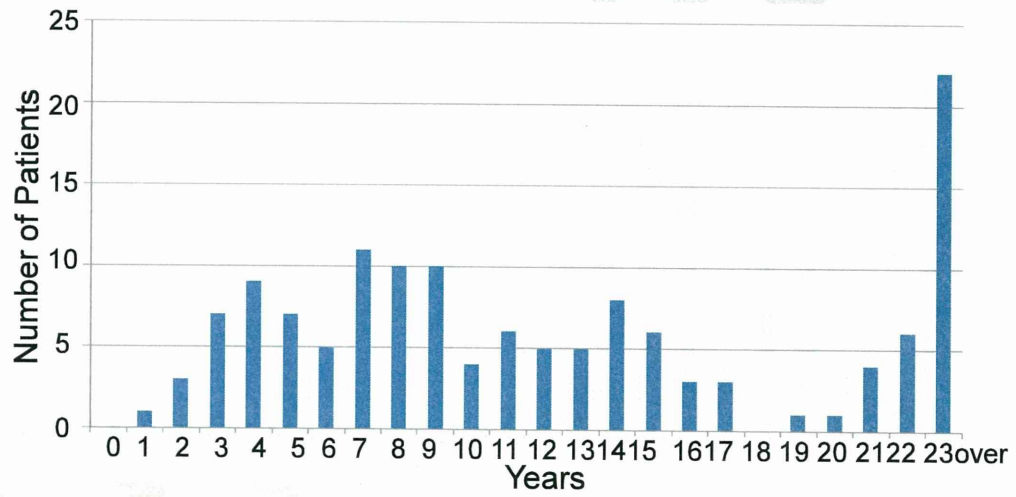


Supplementary Figure 2

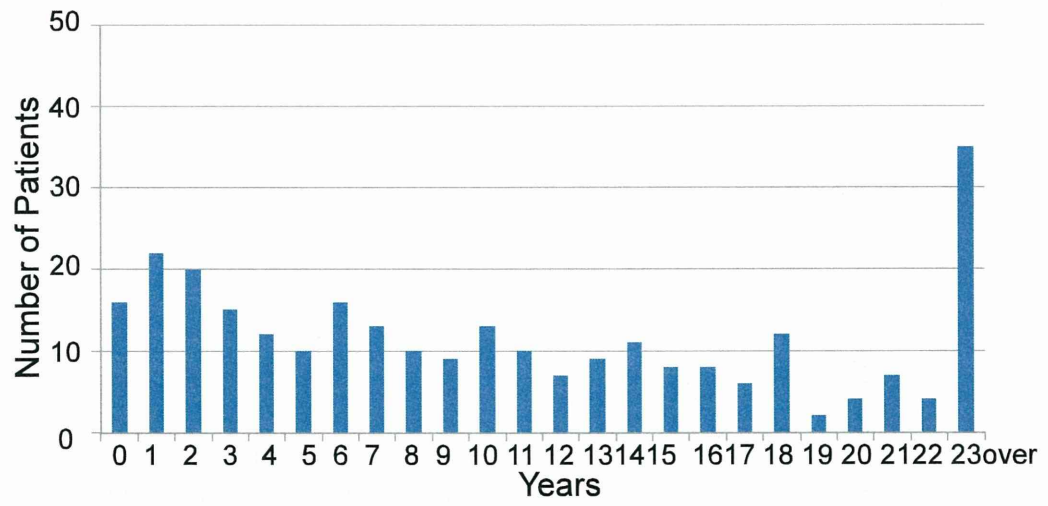
A. BWS



B. AS



C. PWS



D. SRS

