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Supplementary Material

Discovery of human posterior head 20 (hPH20) and homo sapiens sperm acrosome associated 1(hSPACA1) immunocontraceptive epitopes and their effects on fertility in male and female mice

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Fig. S1. (A) PCR identification of biopanning for hSPACA1. The phage library was identificated by PCR after five bio-panning with ASA-positive serum, and the results suggested that Sa and Sc segments could combine with ASA serum antibody and had been panned and identificated. 1 MK; 2, 3, 4, 5, 6: Sa; 7: MK; 8, 9, 10, 11, 12: Sb; 13: MK; 14, 15, 16, 17, 18: Sc. (B) PCR identification of biopanning for hPH20 The phage library was identificated by PCR after five bio-panning with ASA-positive serum, and the results suggested that Pa, Pc and Pd segments could combine with ASA serum antibody and had been panned and identificated.1 MK; 2, 3, 4, 5, 6: Pa; 7: MK; 8, 9, 10, 11, 12: Pb; 13: MK; 14, 15, 16, 17, 18: Pc; 19: MK; 20, 21, 22, 23, 24: Pd; 25 MK.



Fig. S2. Sperm agglutination. The serum was used in the assay after immune Day 65.(A) blank control (B) pre-immune antiserum. Sperm could not been agglutination.All serum of immune-mice were detected, the results showed that every peptide and every mouse serum did not cause sperm agglutination, such as the third female mice

of P82, the second female mice and sixth male mice of Sa6, the fourth male mice of Sa37, and the third female mice and fifth male mice of Sa 76, the antisera could agglutinate sperm (C-H).

Gene fragment	Primer
pTSa	Sense: 5'CAAGAATTCGACCAACGTCACCG 3'
	Antisense:5'GCGAAGATGTGTGAA3'
pTSb	Sense: 5'GAC <u>GAATTC</u> TGGACCAACAGATTG3'
	Antisense: 5'GCCAAGCTTACAGATAATGACTC3'
pTSc	Sense: 5'GTTGAATTCCTTGGCTTTCGAGT3'
	Antisense: 5'GAT <u>AAGCTT</u> TCATTC3'
рТРа	Sense: 5' GACGAATTC TCTGAATTTCAGAGC 3'
	Antisense: 5' CACAAGCTTTTAAGACCTATTCTTG 3'
pTPb	Sense: 5' GAAGAATTCACCCACTTGGGCAAGA 3'
	Antisense: 5' CATAAGCTT TTAGGCTTCCCGAACTC 3'
pTPc	Sense: 5' GAAGAATTC TGTAGCTGCTACACT 3'
	Antisense: 5' CATAAGCTT TTAATCTGGGTTGAGGT 3'
pTPd	Sense: 5' GACGAATTC TATAAGGAAAAACTGG 3'
	Antisense: 5' CACAAGCTTTACAAACTCGCTAC 3'

 Table S1. Primers used for amplification of hPH20 and hSPACA1 gene

	000	Sof	So27	S-76
NT 1	P82	580	5857	5870
Number	18	18	18	1/
MW	2163.5	1870.8	2082.2	1910.1
	8.39	3.77	4.25	4.87
Amino acid				
composition			0.004	0.001
Ala (A)	5.6%	16.7%	0.0%	0.0%
Arg (R)	0.0%	0.0%	5.6%	11.8%
Asn (N)	0.0%	0.0%	11.1%	0.0%
Asp (D)	<mark>16.7%</mark>	5.6%	5.6%	5.9%
Cys (C)	0.0%	0.0%	0.0%	<mark>17.6%</mark>
Gln (Q)	5.6%	5.6%	0.0%	0.0%
Glu (E)	0.0%	<mark>33.3%</mark>	<mark>22.2%</mark>	<mark>17.6%</mark>
Gly (G)	0.0%	<mark>16.7%</mark>	5.6%	5.9%
His (H)	5.6%	5.6%	0.0%	0.0%
Ile (I)	11.1%	0.0%	0.0%	0.0%
Lys	0.0%	0.0%	5.6%	5.9%
Leu (L)	11.1%	5.6%	0.0%	0.0%
Met (M)	0.0%	0.0%	5.6%	0.0%
Phe (F)	5.6%	0.0%	5.6%	0.0%
Pro (P)	0.0%	0.0%	0.0%	5.9%
Ser (S)	5.6%	0.0%	5.6%	5.9%
Thr (T)	5.6%	5.6%	5.6%	5.9%
Trp (W)	0.0%	0.0%	0.0%	0.0%
Tyr (Y)	5.6%	0.0%	0.0%	0.0%
Val (V)	0.0%	5.6%	22.2%	17.6%
Pyl (O)	0.0%	0.0%	0.0%	0.0%
Sec (U)	0.0%	0.0%	0.0%	0.0%
(B)	0.0%	0.0%	0.0%	0.0%
(Z)	0.0%	0.0%	0.0%	0.0%
(X)	0.0%	0.0%	0.0%	0.0%
Total number	3	7	5	4
of negatively				
chargedresidues				
(Asp + Glu)				
Total number	4	0	2	3
of positively				
chargedresidues				
(Arg + Lys)				
Instability	53.79	53.97	36.10	56.34
index				
GRAVY	-0.800	-1.094	-0.739	-0.606

Table S2. ProtParam analyse for peptides

Note: Molecular weight:MW

The P82 peptide has a higher content of Asp (16.7%) and GRAVY -0.800 and does not contain any Trp residues. The Sa6 peptide has a higher content of Glu (33.3%), Gly (16.7%) and GRAVY -1.094 and there are no Trp, Tyr or Cys in the region considered. The Sa37 peptide has higher content of Glu (22.2%) and GRAVY -0.739 and there are no Trp, Tyr or Cys in the region considered. Sa76 has higher content of Cys (17.6%), Glu (17.6%) and GRAVY-0.606 and does not contain any Trp residues.