Supporting Information

Bromfield et al. 10.1073/pnas.1305609111

SI Materials and Methods

Histochemical Analysis of Placental Tissue. Females mated with intact or seminal vesicle-excised (SVX) males were euthanized at 1000–1200 hours on gestation day (gd) 17.5. Placental tissue was dissected free of membranes and fixed in 4% (wt/vol) paraformaldehyde/2.5% polyvinylpyrrolidone (wt/vol) in 70 mM sodium phosphate buffer (pH 7.4) overnight at 4 °C, washed in PBS, and paraffin-embedded. Midsagittal sections (7-μm) were stained with Masson's trichrome, and the area of labyrinthine and junctional tissues was determined by video image analysis with Video Pro software (Leading Edge Software).

Postmortem Body Composition. The following tissues were excised and weighed at postmortem analysis: brain, heart, thymus, kidney (left and right side), adrenal gland (left and right side), liver, spleen, uterus, ovaries (left and right side), testicles (left and right side), parametrial or epididymal fat, retroperitoneal fat, perirenal fat, epididymis, and seminal vesicle glands. The left-side bicep and tricep and the right-side quadricep and gastrocnemius were taken as representative samples of muscle tissue. Total central fat was calculated as the sum of weights of parametrial or epididymal fat, retroperitoneal fat, and perirenal fat. Lean body weight was total body weight minus total central fat. Combined muscle was the sum of weights of the bicep, tricep, quadricep, and gastrocnemius muscles. Biopsies of retroperitoneal fat were processed for histological analysis. After fixation and processing as described above, sections (6-μm) were cut and stained with 0.1% toluidine blue for 15 s and then evaluated by computer-assisted image analysis for adipocyte size.

Blood Pressure, Adipocytokines, and Glucose-Tolerance Test. At 14 wk, resting systolic blood pressure was measured in male progeny using an ML125 noninvasive blood pressure controller and tail cuff (ADInstruments). Following 30 min of acclimatization under restraint at 30 °C, at least five consecutive readings over a 15-min period were taken and averaged. Plasma adiponectin, leptin, and insulin were measured by RIA (Linco Research) in blood drawn just before euthanasia at 0900–1000 hours. The intra- and interassay coefficients of variation were as follows. For insulin, the lowest detectable limit was 0.02 ng/mL, with intraassay precision of <6% and interassay precision of <11%. For adiponectin, the lowest detectable limit was 1 ng/mL, with intraassay precision of <5% and interassay precision of <9%. For leptin, the lowest detectable limit was 0.2 ng/mL, with intraassay precision of <11% and interassay precision of <14%. Glucose and free fatty acids were measured by a Cobas Mira automated centrifugal analyzer (Roche Diagnostic Systems). For i.p. glucose-tolerance tests, a subset of mice was fasted for 12 h and then given 1 mg/g glucose i.p. at 0900 hours. Blood collected via tail cuts 0, 30, and 60 min later was analyzed using a benchtop glucometer (Hemocue). Change in blood glucose was plotted against time, and the area under the curve was calculated.

Reverse Transcription and Quantitative Real-Time PCR. Total cellular RNA was extracted from oviducts on gd 0.5 using TRIzol (Invitrogen) and treated with DNase (DNA-free; Ambion), and then first-strand cDNA was reverse-transcribed from 3 μg random hexamer-primed RNA using a SuperScript III Reverse Transcriptase Kit. Primer pairs specific for published cDNA sequences were designed using Primer Express version 2 software (Applied Biosystems; Table S6). PCR used reagents supplied in a 2× SYBR Green PCR Master Mix (Applied Biosystems), and each reaction (20 μL total) contained 3 μL cDNA and 0.5 μM 5′ and 3′ primers. The negative control contained H_2O substituted for cDNA. PCR amplification was performed in duplicate in an ABI Prism 7000 Sequence Detection System (Applied Biosystems). Reaction products were analyzed by dissociation curve profile and by 2% agarose gel (wt/vol) electrophoresis. Data were normalized independently to *Actb* mRNA expression and plotted as expression relative to the mean of the estrous control group.

In Vivo and in Vitro Embryo Development. To assess embryo numbers and development to two-cell stage, CBAF1 females mated with intact or SVX males were killed at 1000–1200 hours on gd 0.5, and oviducts were excised and flushed with Human Tubal Fluid media supplemented with 0.5% BSA (Sigma-Aldrich) and 2.3 mM Hepes (Sigma-Aldrich) (HTF-Hepes). Total oocytes and oocytes cleaved to two-cell stage were counted. To assess development to blastocyst stage in vitro, two-cell embryos flushed from oviducts on gd 0.5 were cultured in HTF medium, and development to blastocyst stage was scored 3 d later. To assess development to blastocyst stage in vivo, oviducts were excised at 1000–1200 hours on gd 3.5 and flushed with HTF-Hepes. The number and developmental stage of all embryos were scored. Blastocyst-stage embryos were stained with 100 μg/mL propidium iodide and 20 μg/mL Hoechst in HTF-Hepes for 30 min at 37 °C and examined under UV light to count blastomere nuclei.

Embryo Transfers. Prepubertal 3- to 4-wk-old CBAF1 females were given 5 IU pregnant mare serum (Folligon) i.p. at 1200–1300 hours and then 5 IU human CG (Chorulon) i.p. 48 h later, and mated with intact BALB/c males. Two-cell embryos were collected at 0800 hours on gd 1.5 using HTF-Hepes. Morula-stage embryos were flushed from oviducts and uteri at 1700 hours on gd 2.5 using HTF-Hepes and cultured in vitro overnight in HTF medium for transfer 17–18 h later at blastocyst stage. Recipient CBAF1 females (8- to 10-wk-old) were prepared by mating with BALB/c males vasectomized with intact (VAS) or excised seminal vesicles (SVX/VAS). Five embryos were transferred at 0800–1000 hours to each oviduct on gd 0.5 (two cells) or each uterine horn on gd 2.5 (blastocysts) using standard procedures. Pregnancy outcomes were evaluated in two cohorts at gd 17.5 and in offspring at 14 wk.

Statistical Analysis. All data were analyzed using SPSS 12.0 software. To evaluate effects of paternal seminal vesicle excision on parameters in fetuses, progeny, or their tissues, mixed-model ANOVA was used. For analysis of fetal and placental size and preweaning pup weights, mother was the subject and litter size and sex of offspring were covariates. Litter size and cohort were included as covariates in postnatal growth trajectory, adult tissue, and body composition analyses, where interactions were identified using mixed-model ANOVA with the mother as subject. Data expressed as the estimated marginal mean were statistically adjusted to define the number of cases in each group as equal. Categorical data including pregnancy rate, cleavage rate, and developmental stage were analyzed by χ^2 test, and correlations between oviduct cytokine expression and mating treatment were analyzed by Pearson's test. Implantation rate, gestational length, litter size, blastomere number, and quantitative (q)RT-PCR data were compared using one-way ANOVA.

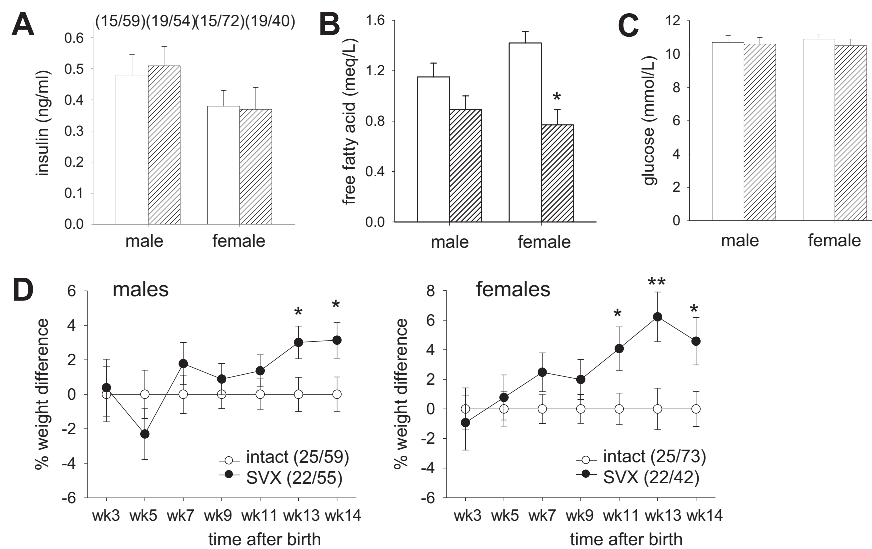


Fig. S1. Seminal vesicle excision alters plasma free fatty acids, but not insulin or glucose, and postnatal growth and weight of male and female adult offspring. In adult male progeny sired by SVX males and evaluated at 14 wk, the plasma content of insulin (*A*), free fatty acid (*B*), and glucose (*C*) was unchanged, compared with control male progeny sired by intact males (*$P < 0.05$). In adult female progeny sired by SVX males, plasma free fatty acid content was reduced (*A*), whereas insulin (*B*) and glucose content (*C*) was unchanged, compared with control female progeny (*$P < 0.05$). Data are the estimated marginal mean ± SEM, and the effect of seminal fluid composition was evaluated by mixed-model ANOVA. Numbers of treated dams and progeny are in parentheses. (*D*) The postweaning growth trajectory of progeny sired by SVX males was altered compared with progeny sired by intact males. Number of dams and progeny are given in parentheses. Data are expressed as the estimated marginal mean ± SEM % weight change, and the effect of seminal fluid composition was evaluated by mixed-model ANOVA, with litter size as covariate. meq/L, molar equivalent per liter. *$P < 0.05$; **$P < 0.01$.

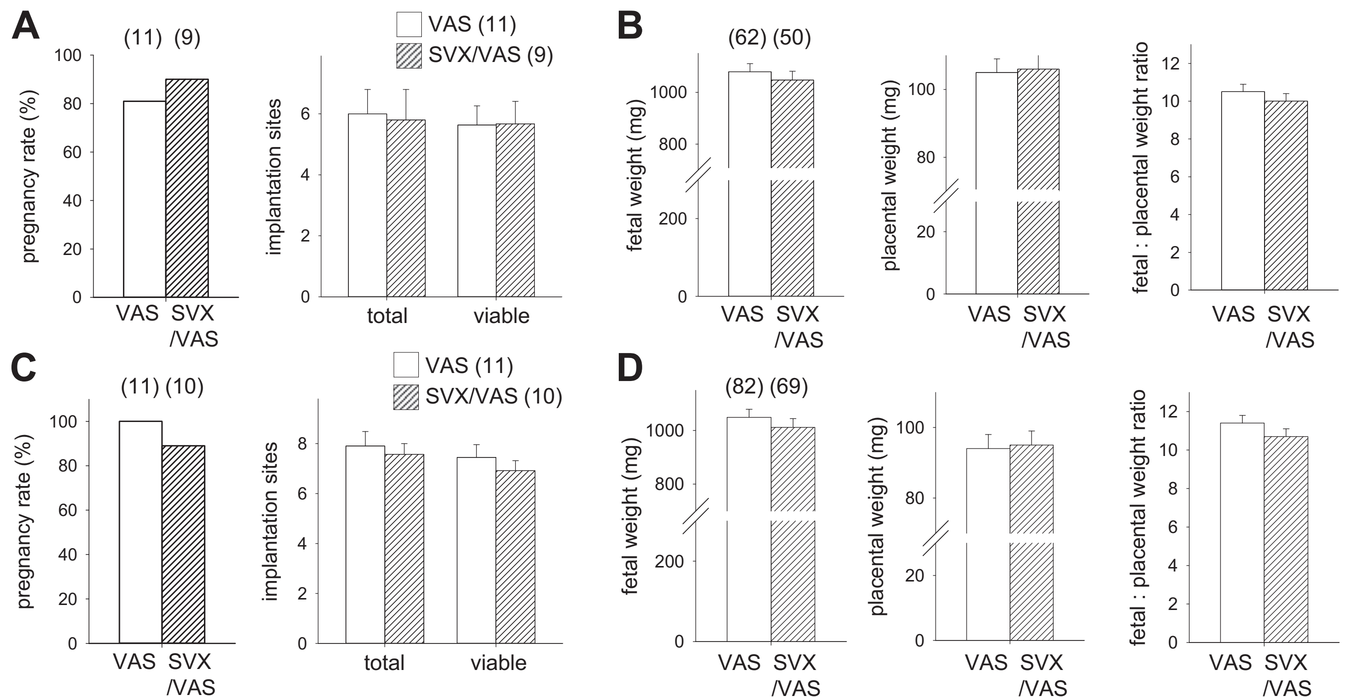
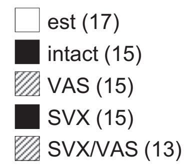


Fig. S2. Recipient exposure to seminal fluid does not impair embryo implantation after two-cell or blastocyst transfer. (*A*) Transfer of two-cell embryos resulted in comparable pregnancy rates, with no change in total or viable implantation sites per recipient at gd 18.5 in recipient females mated with SVX/VAS males, compared with control recipient females mated with VAS males. (*B*) Two-cell embryos transferred to recipient females showed comparable fetal weight, placental weight, and fetal-to-placental weight ratio at gd 18.5 in recipients mated with SVX/VAS males, compared with controls. (*C*) Transfer of blastocyst-stage embryos resulted in comparable pregnancy rates, with no change in total or viable implantation sites per recipient at gd 18.5 in recipient females mated with SVX/VAS males, compared with control recipient females mated with VAS males. (*D*) Blastocyst-stage embryos transferred to recipient females showed comparable fetal weight, placental weight, and fetal-to-placental weight ratio at gd 18.5 in recipients mated with SVX/VAS males, compared with controls. Data are estimated marginal mean ± SEM, and the effect of seminal fluid composition was evaluated by mixed-model ANOVA. Numbers of recipient dams and implantation sites are in parentheses.



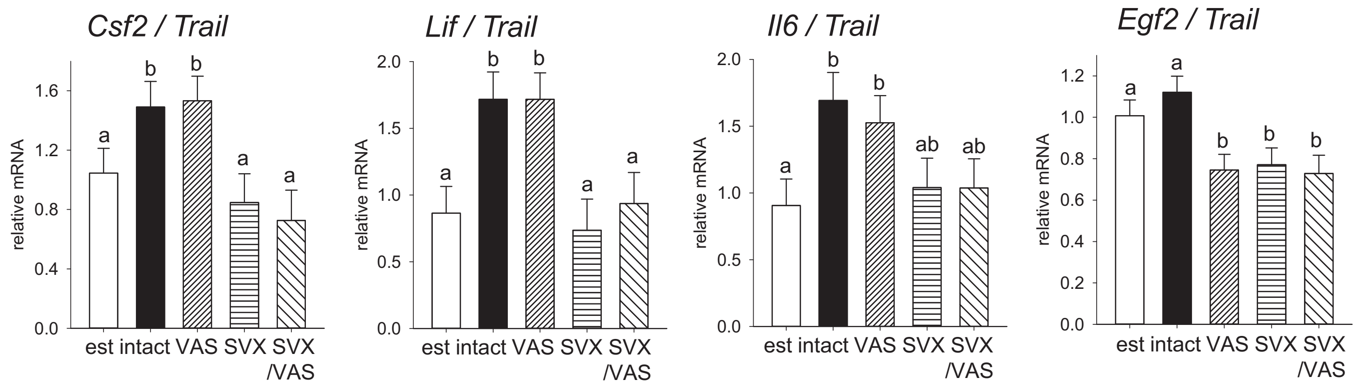


Fig. S3. Seminal vesicle excision inhibits oviduct expression of embryotrophic cytokines after coitus. Oviduct expression of the embryotrophic cytokines *Csf2*, *Lif*, *Il6*, and *Egf* as a ratio to *Trail* expression on gd 0.5 after mating with intact, VAS, SVX, SVX/VAS, or virgin estrous females (est). Data are the estimated marginal mean ± SEM, and the effect of seminal fluid was evaluated by mixed-model ANOVA. Different superscripts represent statistical difference between groups. Numbers of mice are in parentheses.

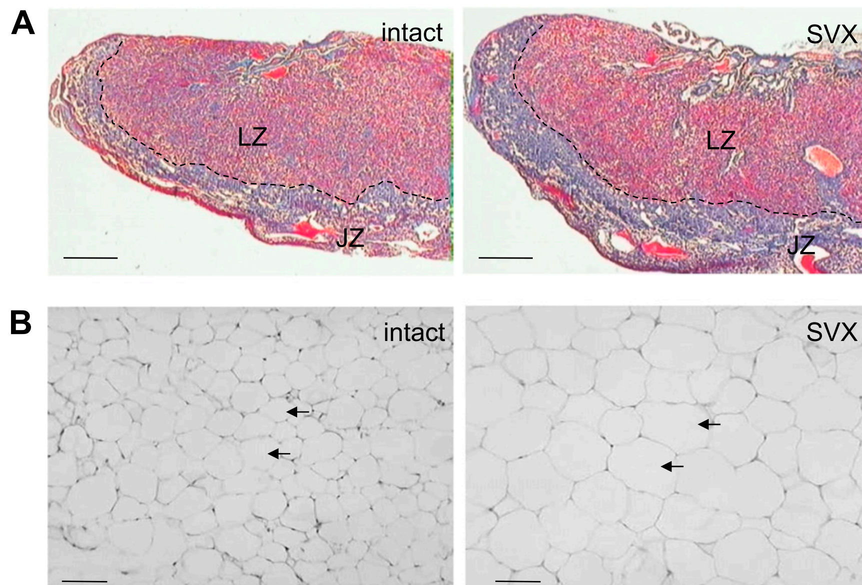


Fig. S4. Seminal vesicle excision alters placental structure in utero and adipocyte size in adult offspring. (*A*) (Large format of photomicrographs from Fig. 1) Total cross-sectional area of midsagittal sections of placentas in pregnancies sired by SVX males, stained with Masson's trichrome, was increased compared with control placentas, with an increase in both the labyrinthine zone (LZ) and junctional zone (JZ). The JZ/LZ boundary is indicated by a dotted line. (Scale bars, 450 μm.) (*B*) (Large format of photomicrographs from Fig. 3). Average adipocyte area in retroperitoneal fat was increased in male but not female progeny of SVX males, compared with controls. Arrows indicate adipocytes. (Scale bars, 50 μm.)

Table S1. Effect of paternal seminal vesicle excision on body morphometry of progeny at 14 wk

Tissue/organ	Intact (relative), %	SVX (relative), %	*P* value	Intact (absolute)	SVX (absolute)	*P* value
Male progeny						
Total body weight, g	—	—	—	31.0 ± 0.7	32.9 ± 0.7	0.082
Lean body weight, g	—	—	—	30.3 ± 0.7	31.7 ± 0.6	NS
Muscle:fat ratio	—	—	—	0.94 ± 0.15	0.49 ± 0.15*	0.047
Total central fat (%), mg‡	2.18 ± 0.33	3.45 ± 0.32*	0.011	685 ± 130	1,179 ± 127*	0.012
Epididymal fat (%), mg	1.62 ± 0.25	2.55 ± 0.24*	0.012	507 ± 98	875 ± 96*	0.013
Retroperitoneal fat (%), mg	0.38 ± 0.06	0.57 ± 0.05*	0.020	121 ± 20	194 ± 19*	0.015
Renal fat (%), mg	0.19 ± 0.31	0.32 ± 0.03*	0.010	58 ± 13	109 ± 13*	0.013
Combined muscle (%), mg§	1.39 ± 0.06	1.27 ± 0.06	NS	422 ± 20	418 ± 20	NS
Gastrocnemius muscle (%), mg	0.39 ± 0.03	0.36 ± 0.03	NS	131 ± 8	119 ± 9	NS
Quadricep muscle (%), mg	0.52 ± 0.03	0.47 ± 0.03	NS	169 ± 8	166 ± 7	NS
Bicep muscle (%), mg	0.06 ± 0.03	0.06 ± 0.03	NS	19 ± 1	18 ± 1	NS
Tricep muscle (%), mg	0.35 ± 0.02	0.36 ± 0.02	NS	109 ± 6	116 ± 5	NS
Brain (%), mg	1.33 ± 0.03	1.35 ± 0.03	NS	413 ± 9	437 ± 9	0.076
Heart (%), mg	0.50 ± 0.01	0.52 ± 0.01	NS	154 ± 5	171 ± 5*	0.024
Liver (%), mg	5.10 ± 0.10	5.09 ± 0.09	NS	1,595 ± 54	1,668 ± 52	NS
Kidney (L+R) (%), mg	1.57 ± 0.04	1.58 ± 0.04	NS	489 ± 18	517 ± 18	NS
Lungs (%), mg	0.56 ± 0.01	0.56 ± 0.01	NS	172 ± 4	183 ± 4	0.059
Spleen (%), mg	0.24 ± 0.02	0.25 ± 0.01	NS	81 ± 3	84 ± 3	NS
Seminal vesicle (%), mg	0.69 ± 0.03	0.71 ± 0.02	NS	215 ± 10	231 ± 8	NS
Testes (L+R) (%), mg	0.71 ± 0.03	0.71 ± 0.03	NS	221 ± 6	233 ± 6	NS
Female progeny						
Total body weight, g	—	—	—	25.7 ± 0.7	25.5 ± 0.6	NS
Lean body weight, g	—	—	—	24.9 ± 0.6	24.7 ± 0.6	NS
Muscle:fat ratio	—	—	—	0.57 ± 0.11	0.50 ± 0.10	NS
Total central fat (%), mg‡	3.10 ± 0.35	2.90 ± 0.31	NS	808 ± 97	762 ± 88	NS
Parametrial fat (%), mg	2.31 ± 0.27	2.14 ± 0.24	NS	603 ± 76	564 ± 68	NS
Retroperitoneal fat (%), mg	0.30 ± 0.03	0.28 ± 0.03	NS	78 ± 10	74 ± 9	NS
Renal fat (%), mg	0.48 ± 0.05	0.47 ± 0.06	NS	128 ± 14	124 ± 13	NS
Combined muscle (%), mg§	1.39 ± 0.06	1.24 ± 0.05†	0.052	359 ± 16	312 ± 12*	0.025
Gastrocnemius muscle (%), mg	0.43 ± 0.02	0.37 ± 0.02	0.062	106 ± 5	93 ± 5†	0.057
Quadricep muscle (%), mg	0.55 ± 0.02	0.49 ± 0.02	0.083	136 ± 6	125 ± 6	NS
Bicep muscle (%), mg	0.07 ± 0.01	0.06 ± 0.01	NS	13 ± 1	14 ± 1	NS
Tricep muscle (%), mg	0.35 ± 0.02	0.34 ± 0.02	NS	82 ± 4	87 ± 4	NS
Brain (%), mg	1.68 ± 0.04	1.81 ± 0.04*	0.023	411 ± 8	460 ± 8**	<0.001
Heart (%), mg	0.48 ± 0.01	0.53 ± 0.01*	0.016	126 ± 5	134 ± 4	NS
Liver (%), mg	5.06 ± 0.11	5.18 ± 0.09	NS	1,309 ± 47	1,322 ± 37	NS
Kidney (L+R) (%), mg	1.26 ± 0.03	1.30 ± 0.02	NS	325 ± 11	333 ± 8	NS
Lungs (%), mg	0.60 ± 0.05	0.64 ± 0.04	NS	146 ± 8	169 ± 9	NS
Spleen (%), mg	0.35 ± 0.01	0.35 ± 0.01	NS	86 ± 3	88 ± 3	NS
Uterus (%), mg	0.52 ± 0.05	0.52 ± 0.05	NS	133 ± 11	131 ± 12	NS
Ovary (L+R) (%), mg	0.07 ± 0.01	0.05 ± 0.01	NS	13 ± 1	13 ± 1	NS

Data are from $n = 16$ male progeny and $n = 17$ female progeny from 15 litters sired by intact males, and $n = 19$ male progeny and $n = 18$ female progeny from 14 litters sired by SVX males. Data are expressed as the estimated marginal mean ± SEM. Relative or absolute weight and effect of seminal fluid composition were compared by mixed-model linear repeated-measures ANOVA, using litter size and cohort as covariates when identified as significant. P values are shown when $P < 0.1$. †$P < 0.06$; *$P < 0.05$; **$P < 0.01$ compared with intact mating group. L, left; NS, not significant; R, right.

‡Total central fat is the sum of all fat depots measured.

§Combined muscle is the sum of all muscles measured.

Table S2. Effect of recipient female seminal plasma exposure on body morphometry of progeny at 14 wk after two-cell transfer

Tissue/organ	VAS (relative), %	SVX/VAS (relative), %	*P* value	VAS (absolute)	SVX/VAS (absolute)	*P* value
Male progeny						
Total body weight, g	—	—	—	32.1 ± 0.5	32.3 ± 0.5	NS
Lean body weight, g	—	—	—	31.4 ± 0.4	31.4 ± 0.5	NS
Muscle:fat ratio	—	—	—	0.61 ± 0.06	0.58 ± 0.07	NS
Total central fat (%), mg[‡]	2.33 ± 0.22	2.98 ± 0.24*	0.050	749 ± 78	981 ± 87[†]	0.054
Epididymal fat (%), mg	1.71 ± 0.17	2.23 ± 0.19*	0.046	550 ± 60	733 ± 66*	0.048
Retroperitoneal fat (%), mg	0.40 ± 0.04	0.49 ± 0.04	0.096	128 ± 13	161 ± 13	0.098
Renal fat (%), mg	0.22 ± 0.02	0.26 ± 0.02	NS	71 ± 7	87 ± 7	NS
Combined muscle (%), mg[§]	1.29 ± 0.05	1.39 ± 0.06	NS	415 ± 18	449 ± 19	NS
Gastrocnemius muscle (%), mg	0.36 ± 0.03	0.44 ± 0.03*	0.038	113 ± 8	143 ± 9*	0.027
Quadricep muscle (%), mg	0.53 ± 0.03	0.54 ± 0.03	NS	169 ± 9	174 ± 1	NS
Bicep muscle (%), mg	0.05 ± 0.01	0.05 ± 0.01	NS	16 ± 1	17 ± 1	NS
Tricep muscle (%), mg	0.36 ± 0.01	0.36 ± 0.01	NS	116 ± 4	115 ± 4	NS
Brain (%), mg	1.43 ± 0.03	1.38 ± 0.03	NS	458 ± 7	443 ± 7	NS
Heart (%), mg	0.54 ± 0.01	0.51 ± 0.01	0.062	173 ± 4	164 ± 4	NS
Liver (%), mg	4.96 ± 0.06	4.85 ± 0.07	NS	1,591 ± 30	1,569 ± 34	NS
Kidney (L+R) (%), mg	1.60 ± 0.04	1.56 ± 0.04	NS	513 ± 14	504 ± 16	NS
Lungs (%), mg	0.58 ± 0.02	0.56 ± 0.02	NS	187 ± 5	181 ± 6	NS
Spleen (%), mg	0.26 ± 0.01	0.26 ± 0.01	NS	82 ± 3	84 ± 3	NS
Seminal vesicle (%), mg	0.64 ± 0.04	0.62 ± 0.05	NS	206 ± 13	200 ± 15	NS
Testes (L+R) (%), mg	0.72 ± 0.02	0.72 ± 0.02	NS	232 ± 5	232 ± 6	NS
Female progeny						
Total body weight, g	—	—	—	25.6 ± 0.5	25.0 ± 0.5	NS
Lean body weight, g	—	—	—	24.7 ± 0.5	24.1 ± 0.4	NS
Muscle:fat ratio	—	—	—	0.46 ± 0.07	0.51 ± 0.06	NS
Total central fat (%), mg[‡]	3.13 ± 0.36	3.24 ± 0.35	NS	824 ± 107	825 ± 104	NS
Parametrial fat (%), mg	2.28 ± 0.27	2.33 ± 0.26	NS	600 ± 80	593 ± 78	NS
Retroperitoneal fat (%), mg	0.35 ± 0.05	0.34 ± 0.05	NS	93 ± 15	86 ± 14	NS
Renal fat (%), mg	0.50 ± 0.05	0.57 ± 0.05	NS	131 ± 15	146 ± 15	NS
Combined muscle (%), mg[§]	1.14 ± 0.05	1.36 ± 0.04**	0.001	290 ± 12	335 ± 12*	0.012
Gastrocnemius muscle (%), mg	0.30 ± 0.02	0.41 ± 0.02**	0.004	78 ± 6	100 ± 6*	0.018
Quadricep muscle (%), mg	0.46 ± 0.02	0.55 ± 0.02*	0.014	118 ± 6	136 ± 6[†]	0.052
Bicep muscle (%), mg	0.05 ± 0.01	0.05 ± 0.01	NS	12 ± 0	13 ± 0	NS
Tricep muscle (%), mg	0.33 ± 0.01	0.35 ± 0.01	NS	82 ± 3	87 ± 3	NS
Brain (%), mg	1.77 ± 0.04	1.81 ± 0.03	NS	451 ± 8	449 ± 7	NS
Heart (%), mg	0.52 ± 0.01	0.53 ± 0.01	NS	131 ± 3	130 ± 3	NS
Liver (%), mg	5.16 ± 0.09	4.92 ± 0.09[†]	0.065	1,315 ± 32	1,226 ± 31*	0.05
Kidney (L+R) (%), mg	1.28 ± 0.04	1.22 ± 0.03	NS	327 ± 11	304 ± 10	NS
Lungs (%), mg	0.67 ± 0.02	0.70 ± 0.02	NS	170 ± 5	175 ± 5	NS
Spleen (%), mg	0.35 ± 0.01	0.36 ± 0.01	NS	89 ± 3	91 ± 3	NS
Uterus (%), mg	0.47 ± 0.04	0.52 ± 0.04	NS	117 ± 10	129 ± 9	NS
Ovary (L+R) (%), mg	0.05 ± 0.01	0.06 ± 0.01[†]	0.065	12 ± 1	15 ± 1[†]	0.054

Data are from $n = 21$ male progeny and $n = 18$ female progeny from 12 litters after two-cell embryo transfer to recipients prepared by mating with VAS males, and $n = 17$ male progeny and $n = 19$ female progeny from 10 litters from recipients prepared by mating with SVX/VAS males. Data are expressed as the estimated marginal mean ± SEM. Relative or absolute weight and effect of seminal fluid composition were compared by mixed-model linear repeated-measures ANOVA. Litter size was not a significant covariate. *P* values are shown when $P < 0.1$. [†]$P < 0.06$; *$P < 0.05$; **$P < 0.01$ compared with VAS mating group.

[‡]Total central fat is the sum of all fat depots measured.

[§]Combined muscle is the sum of all muscles measured.

Table S3. Effect of embryo transfer versus natural mating on plasma metabolic hormones

Hormone	Natural mating (n = 32)	Two-cell transfer (n = 22)	Blastocyst transfer (n = 18)
Leptin, ng/mL	5.5 ± 0.4^{a}	7.0 ± 0.4^{b}	$7.3 \pm 0.7^{a,b}$
Adiponectin, ng/mL	6.0 ± 0.2^{a}	5.1 ± 0.2^{b}	6.6 ± 0.4^{a}
Leptin:adiponectin ratio	1.1 ± 0.1^{a}	1.5 ± 0.1^{b}	$1.3 \pm 0.2^{a,b}$

In adult progeny at 14 wk, plasma leptin, adiponectin, and leptin:adiponectin ratio were influenced by mode of conception. Data are the estimated marginal mean ± SEM, and the effect of mode of conception was evaluated by mixed-model ANOVA with sex as a covariate. Different superscripts represent statistical significance between groups ($P < 0.05$). Numbers of mated dams are in parentheses. When progeny from both embryo transfer groups were combined and compared with progeny of natural mating, there was a significant increase in leptin ($P = 0.005$), a significant decrease in adiponectin ($P = 0.034$), and a significant increase in the leptin: adiponectin ratio ($P = 0.002$).

Table S4. Effect of recipient female seminal plasma exposure on body morphometry of progeny at 14 wk after blastocyst transfer

Tissue/organ	VAS (relative), %	SVX/VAS (relative), %	P value	VAS (absolute)	SVX/VAS (absolute)	P value
Male progeny						
Total body weight, g	—	—	—	34.3 ± 1.0	34.5 ± 1.0	NS
Lean body weight, g	—	—	—	32.9 ± 1.0	33.2 ± 1.0	NS
Muscle:fat ratio	—	—	—	0.42 ± 0.12	0.48 ± 0.12	NS
Total central fat (%), mg[†]	4.10 ± 0.61	3.67 ± 0.61	NS	1,411 ± 220	1,271 ± 220	NS
Epididymal fat (%), mg	3.14 ± 0.48	2.74 ± 0.48	NS	1,079 ± 172	952 ± 172	NS
Retroperitoneal fat (%), mg	0.64 ± 0.09	0.59 ± 0.09	NS	221 ± 30	202 ± 30	NS
Renal fat (%), mg	0.32 ± 0.06	0.34 ± 0.06	NS	111 ± 23	118 ± 23	NS
Combined muscle (%), mg[‡]	1.23 ± 0.09	1.33 ± 0.09	NS	416 ± 31	457 ± 31	NS
Gastrocnemius muscle (%), mg	0.42 ± 0.05	0.41 ± 0.05	NS	141 ± 17	142 ± 17	NS
Quadricep muscle (%), mg	0.50 ± 0.04	0.50 ± 0.04	NS	166 ± 12	173 ± 12	NS
Bicep muscle (%), mg	0.05 ± 0.01	0.06 ± 0.01	NS	17 ± 1	20 ± 1	NS
Tricep muscle (%), mg	0.27 ± 0.03	0.35 ± 0.03*	0.033	91 ± 9	122 ± 9*	0.029
Brain (%), mg	1.23 ± 0.04	1.21 ± 0.04	NS	423 ± 15	416 ± 15	NS
Heart (%), mg	0.49 ± 0.02	0.51 ± 0.02	NS	167 ± 7	174 ± 7	NS
Liver (%), mg	5.17 ± 0.13	5.16 ± 0.13	NS	1,776 ± 83	1,785 ± 83	NS
Kidney (L+R) (%), mg	1.64 ± 0.05	1.55 ± 0.05	NS	561 ± 23	535 ± 23	NS
Lungs (%), mg	0.52 ± 0.02	0.50 ± 0.02	NS	177 ± 6	174 ± 6	NS
Spleen (%), mg	0.22 ± 0.03	0.28 ± 0.03	NS	75 ± 10	98 ± 10	NS
Seminal vesicle (%), mg	0.66 ± 0.04	0.65 ± 0.04	NS	222 ± 10	222 ± 10	NS
Testes (L+R) (%), mg	0.70 ± 0.03	0.69 ± 0.03	NS	236 ± 6	235 ± 6	NS
Female progeny						
Total body weight, g	—	—	—	27.7 ± 0.8	26.6 ± 0.7	NS
Lean body weight, g	—	—	—	26.5 ± 0.7	25.7 ± 0.6	NS
Muscle:fat ratio	—	—	—	0.34 ± 0.08	0.42 ± 0.08	NS
Total central fat (%), mg[†]	4.27 ± 0.47	3.31 ± 0.45	NS	1,212 ± 150	892 ± 141	NS
Parametrial fat (%), mg	3.14 ± 0.34	2.38 ± 0.32	NS	887 ± 108	642 ± 102	NS
Retroperitoneal fat (%), mg	0.43 ± 0.06	0.36 ± 0.06	NS	123 ± 19	96 ± 18	NS
Renal fat (%), mg	0.72 ± 0.08	0.58 ± 0.08	NS	202 ± 25	154 ± 23	NS
Combined muscle (%), mg[‡]	1.15 ± 0.07	1.23 ± 0.07	NS	315 ± 15	327 ± 14	NS
Gastrocnemius muscle (%), mg	0.41 ± 0.05	0.38 ± 0.04	NS	114 ± 11	102 ± 10	NS
Quadricep muscle (%), mg	0.43 ± 0.03	0.50 ± 0.03	0.062	118 ± 8	133 ± 7	NS
Bicep muscle (%), mg	0.05 ± 0.01	0.06 ± 0.07	NS	14 ± 1	16 ± 1	NS
Tricep muscle (%), mg	0.25 ± 0.02	0.29 ± 0.02	NS	69 ± 5	75 ± 5	NS
Brain (%), mg	1.41 ± 0.05	1.67 ± 0.05**	0.003	391 ± 13	443 ± 13*	0.012
Heart (%), mg	0.47 ± 0.03	0.49 ± 0.03	NS	130 ± 6	131 ± 5	NS
Liver (%), mg	5.00 ± 0.17	5.25 ± 0.16	NS	1,385 ± 69	1,405 ± 65	NS
Kidney (L+R) (%), mg	1.29 ± 0.05	1.35 ± 0.05	NS	353 ± 11	359 ± 10	NS
Lungs (%), mg	0.57 ± 0.03	0.62 ± 0.03	NS	157 ± 6	166 ± 6	NS
Spleen (%), mg	0.33 ± 0.01	0.38 ± 0.01*	0.011	89 ± 4	100 ± 4	0.092
Uterus (%), mg	0.44 ± 0.04	0.43 ± 0.04	NS	121 ± 11	113 ± 10	NS
Ovary (L+R) (%), mg	0.05 ± 0.01	0.06 ± 0.01	NS	13 ± 2	14 ± 2	NS

Data are from $n = 9$ male progeny and $n = 8$ female progeny from 9 litters after blastocyst transfer to recipients prepared by mating with VAS males, and $n =$ 9 male progeny and $n = 9$ female progeny from 9 litters from recipients prepared by mating with SVX/VAS males. Data are expressed as the estimated marginal mean ± SEM. Relative or absolute weight and effect of seminal fluid composition were compared by mixed-model linear repeated-measures ANOVA. Litter size was not a significant covariate. P values are shown when $P < 0.1$. *$P < 0.05$; **$P < 0.01$ compared with VAS mating group.

[†]Total central fat is the sum of all fat depots measured.

[‡]Combined muscle is the sum of all muscles measured.

Table S5. Determinants of oviduct cytokine expression

Cytokine mRNA	Mating	Seminal plasma	Sperm	Zygote
*Csf2**	0.124, NS[†]	0.423, $P < 0.001$	−0.030, NS	0.170, NS
Lif	0.107, NS	0.440, $P < 0.001$	−0.066, NS	0.219, NS
Il6	0.135, NS	0.320, $P = 0.008$	0.040, NS	0.152, NS
Egf	−0.238, $P = 0.041$	0.005, NS	0.130, NS	0.254, $P = 0.029$
Trail	−0.180, NS	−0.253, $P = 0.033$	−0.222, NS	−0.309, $P = 0.009$

*Oviductal expression of the cytokines *Csf2*, *Lif*, *Il6*, *Egf*, and *Trail* was quantified by qRT-PCR on gd 0.5 after mating with intact ($n = 15$), VAS ($n = 15$), SVX ($n = 15$), SVX/VAS ($n = 13$), or virgin estrous females ($n = 17$). Data according to treatment group are shown in Fig. 6. The correlation between mRNA expression and factors including mating (all four mated groups), exposure to seminal plasma (VAS and SVX/VAS), exposure to sperm (intact and SVX), or presence of zygotes (intact) was determined by correlation analysis.
[†]Pearson's correlation coefficients and *P* values are shown.

Table S6. Primer sequences for qRT-PCR

Cytokine mRNA	5′ primer	3′ primer	Accession no.	Product size (bp)
Csf2	CCTGGGCATTGTGGTCTACAG	GGCATGTCATCCAGGAGGTT	X03019	117
Lif	CGCCAATGCTCTCTTCATTTC	TCCGATACAGCTCCACCAACT	NM_008501	113
Il6	ACAACCACGGCCTTCCCTAC	TCCACGATTTCCCAGAGAACA	NM_031168	228
Egf	CCCAGCGAGAAAGACTGATCA	CAGATGGCTCCCTCCAACAA	NM_010113	122
Trail	CCAGAGATGCCGAGTACGGA	AAGGCTCCAAAGAAGCTGGCT	NM_009425	139
Actb	TGTGATGGTGGGTATGGGTC	ACACGCAGCTCATTGTA	NM_007393.3	162