SUPPORTING MATERIAL

Reproductive Manipulations to Re-establish HA Sheep:

MOET: Ewes in the first experiments [30,31] were synchronized for use as embryo donors for by means of Cervical Implant Drug Release (CIDR) for 14 days and superovulated with declining doses of follicle-stimulating hormone (FSH) (184mg in 1st set of experiments, increased to 228mg in subsequent experiments) twice daily for 3 days. Pregnant mare's serum gonadotropin *(*PMSG) (200IU in 1st set of experiments, increased to 500mg in subsequent experiments) was given with the final dose of FSH and 1000IU of human chorionic gonadotropin *(*hCG*)* 12h post-CIDR removal. The ewes were surgically inseminated 24h later at the uterotubal junction with approximately 1–2.0×10^6 spermatozoa. Oviducts were flushed 40-48h post-insemination with warm M199 containing Hanks salts, 25 mm HEPES, 10% FBS, and 0.5 μg mL^{-1} gentamicin. The resultant embryos were transferred surgically to the oviducts of recipients that had been synchronized using sponges (Ovakron; Heriot Agvet, Rowville, Victoria, Australia) containing 30mg of flugestone acetate (14 days) and given PMSG (400IU in 1st set of experiments, increased to 600mg in subsequent experiments) at sponge removal, followed by 1000IU of hCG 12h post-sponge removal. Implanted ewes were then allowed to complete pregnancy without further intervention.

ICSI: Based on poor initial results with MOET on the first straw of thawed semen, in our first round of experiments to re-establish HA carriers, spermatozoa were also used for intracytoplasmic sperm injection (ICSI) [32]. To accomplish this, ewes were synchronized with CIDR (15 days) and superovulated with a declining dose of FSH (204 mg) twice daily for 3.5 days. Oocytes were then collected and used for ICSI. Utilizing

236 oocytes, ICSI produced 189 embryos, which were implanted into recipients that had been synchronized as detailed above for MOET.

IVF: In our second round of experiments to create hemophiliacs by backcrossing carrier females with semen from an affected male, semen was also used to produce embryos via in vitro fertilization (IVF). Semen for IVF was prepared by centrifugation on a Percoll gradient. As detailed above for ICSI, oocytes were collected via follicular aspiration during midventral laparotomy from superstimulated ewes (as above, but with an increase of FSH to 252 mg). Oocytes and sperm were incubated in mTALP with 20% estrus sheep serum (modified from Bavister et al. 1977 Bio. Reprod. 16, 228–237) for 20 h, then vortexed to remove cumulus cells, and cultured in G1.3 medium (Vitrolife, Englewood, CO) with BSA until transfer. Over the course of our two rounds of experiments, a total of 387 oocytes were used for IVF, producing 146 embryos, which were transferred, at 24 to 48h post-fertilization, to recipients that had been synchronized as detailed above for MOET.

SUPPLEMENTAL TABLE 1

Primers Used to Reverse Transcribe Sheep FVIII mRNA

exon 7 RT	5' - TGG TGG GAA GAG ATA CGA CA - 3'
exon 8 rev	5'- AAG GGA GGC ACA CTG TCA CC- 3'
exon 14b rev	5' -GGG AGT TCT TGC CAT GGG TCC T- 3'
exon 14c rev	5' -TCA TCT CTT CTA GGC TGG TGT CC- 3'
exon 16 rev	5'- CTT GGA GGG AAT CTT TTC AGA GC- 3'
exon 25 rev	5'- GCC ATG TTT TCG GCA AAG TAC C- 3'
14-26 rev	5' - TGC AGT CAA TGG GAA AAG AA - 3'
oligo dT	Qiagen Oligo dT

SUPPLEMENTAL TABLE 2

Primers Used to Clone Sheep Wild Type (WT) and Hemophiliac (H) FVIII Fragments for Sequencing

Gene	Position	Primer Sequence
WT-FVIII	5'UTR	fwd 5'- GCC ATC AGA GCC ATG CAC -3'
WT-FVIII	5'UTR	rev 5'- TGC CAA CTT TTC CCT TCA TC -3'
WT-FVIII	exon1-6	fwd 5'- CCT CGG TGC AGT GGA ACT GTC G -3'
WT-FVIII	exon1-6	rev 5'- GTC CAT CAG GAC TGT CTG AGC GGT AA -3
WT-FVIII	exon4-14	fwd 5'- CCA AAG GGA GAA GGA AGA TG -3'
WT-FVIII	exon4-14	rev 5' - GGG AGT TCT TGC CAT GGG TCC T -3'
WT-FVIII	exon14-26	fwd 5'- TTA GGT GCC ATT GTA TTT GGC- 3'
WT-FVIII	exon14-26	rev 5'- TGC AGT CAA TGG GAA AAG AA -3'
H FVIII	5'UTR	fwd 5'- GCC ATC AGA GCC ATG CAC -3'
H FVIII	5'UTR	rev 5'- TGC CAA CTT TTC CCT TCA TC -3'
H FVIII	exon1-6	fwd 5'- CCT CGG TGC AGT GGA ACT GTC G -3'
H FVIII	exon1-6	rev 5'- GTC CAT CAG GAC TGT CTG AGC GGT AA -3
H FVIII	exon4-8	fwd 5'- CCA AAG GGA GAA GGA AGA TG -3'
H FVIII	exon4-8	rev 5'- AAG GGA GGC ACA CTG TCA CC -3'
H FVIII	exon8-14	fwd 5'- TGT CGT ATC TCT TCC CAC CA -3'
H FVIII	exon8-14	rev 5'- GCC AAA TAC AAT GGC ACC TAA -3'
H FVIII	exon13-14	fwd 5'- GGC ACA TCT TGG ACC AGA GC -3'
H FVIII	exon13-14	rev 5' - GGG AGT TCT TGC CAT GGG TCC T -3'
H FVIII	H16a	fwd 5'- TTA GGT GCC ATT GTA TTT GGC- 3'
H FVIII	H16a	rev 5'- CAA GTC CCT GTG TTT TCT GAA GG -3'
H FVIII	H25a	fwd 5'- AGC ACC CTG GGG AAA CAG G -3'
H FVIII	H25a	rev 5'- CAC ACG GCA GAT CAG AAT GG -3'
H FVIII	H24	fwd 5'- CAT TCT GAT CTG CCG TGT GG -3'
H FVIII	H24	rev 5'- CACAGAGGCATCGAAATACAGC -3'
H FVIII	H26	fwd 5'- CCC ACT GTT TAC CCG CTT CC -3'
H FVIII	H26	rev 5'- TGC AGT CAA TGG GAA AAG AA -3'