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Excessive rumen protected choline in daily diet compromises sperm quality as a result of aberrant DNA methylation modification

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SUPPLEMENTARY MATERIAL

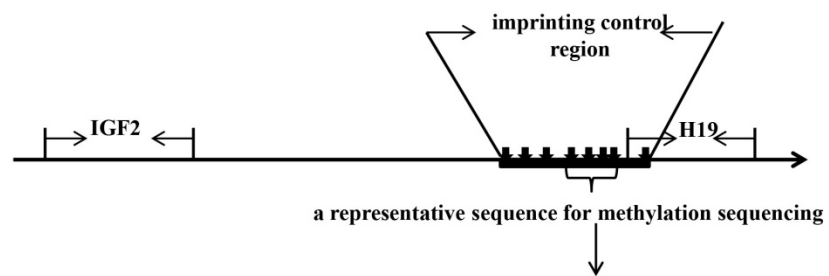
Supplementary Table 1. Daily diet composition and nutrient levels of the basal diet (air-dry basis)⁽¹⁾ (%)

Feedstuffs	Ingredients	Content	Nutrient levels	Content
Coarse fodder (30%)	corn straw silage	60	DM	97.9
	alfalfa hay	40	ME/(MJ/kg)	8.89
Refined fodder (70%)	Corn	60	CP	15.2
	Soybean meal	11.5	EE	3.38
	Bran	12.5	NDF	44.28
	Cottonseed meal	9.8	ADF	28.31
	NaHCO ₃	1.6	Ca	1.15
	Limestone	1.2	P	0.53
	NaCl	1.6		
	Premix ⁽²⁾	1.8		
	Total	100.0		

(1) Total choline content in basal diet was 303.6±21.3 mg/kg.

(2) One kg of premix ingredients are as follows: FeSO₄·7H₂O 180 g, CuSO₄·5H₂O 80 g, MnSO₄·5H₂O 260 g, CoCl₂·6H₂O 490 mg, KI 210 mg, Na₂ SeO₃ 120 mg, VA 650 000 IU, VE 510 IU, VB12 1.0 mg.

Supplementary Figure 1. The typical imprinting control region (ICR) in IGF2 and H19 genes



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CACGGGCGAGACCAGGCTAGGCGGGGCGGGTGGGGGTGGCGTGAGTGTGCACTCTCGGGG
GAAGGGAGGTCAGGGCACAGGCAGGGGCGAGGGGCCGGAGCCGGGCACCGAGCCAGTGG
GTGGGGCACTGGACTTTGAGCCCGTGGCCAAGGCGGGCCTCTGTGGGCGATGACGAAGCG
GGATCGGTGCCTTTGAGCTTGGACCGGAGACGAAGGTGAGTGCCAGCCCGCT
    
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The imprinting control region(ICR) between IGF2 and H19 containing eight methylation islands in total, a representative sequence encompassing twenty CpG sites located four methylation islands concentrated together was used for methylation sequencing.

Supplementary Table 2. Primers for RT-qPCR

Genes (Gene ID)	Primer sequences (5'-3')	T _m (°C)	Product Length (bp)
DNMT 1 (100861294)	Forward 5'- CAGAGAACGAGTTGCTGGGC-3' Reverse 5'- ATGTGGAGCAGCAAGCAGGC-3'	56	278
DNMT 3a (102190884)	Forward 5'- AGCAGAGGGAGAGGGTGCCA-3' Reverse 5'- CCACACGTCCCGCTTGCGCTTG-3'	56	150
DNMT 3b (GAOF01054237)	Forward 5'- CTGGAGGCATCGGCACCCCTA-3' Reverse 5'- CTGGGCTTTCAGACCGAGTCA-3'	56	179
DNMT 3l (102170557)	Forward 5'- CATTACGAAACTACCTT-3' Reverse 5'-CTGCTTGCTGATCCACAT-3'	55	201
<i>IGF2</i> (559098474)	Forward 5'-GATGTCGGTGCCTCTCATG-3' Reverse 5'-AGTACGACGTCGTGCATCTCG-3'	55	205
H19 (162424987)	Forward 5'-AGGTGCTCAAGGGCTACGACT-3' Reverse 5'-TGGCGTTGGGCGAGATGTGC-3'	56	251
β-Aactin (393804138)	Forward 5'-CTCACCTTCTTGGCCTTCG-3' Reverse 5'-GGAAACAGCACTCCTCAACG-3'	56	243

Appendix 1: Cost estimation

1. The cost of rumen protected choline (RPC) consumed by each ram

- (1) Each ram consumes 1.0 kg of concentrate feed per day, and RPC is added at the rate of 0.5%.
- (2) The price of RPC is 65.0 RMB/kg, with a purity of 50%
- (3) The daily cost is: $1.0 \times 0.5\% \times 65.0 = 0.325$ RMB / day
- (4) RPC cost before ram maturity is: $0.325 \times 22 \times 7 = 50.05$ RMB
- (5) A reproductive cycle is the sum of 155 days of gestation and 42 days of interval
- (6) The cost of each breeding cycle of each ram is: $0.325 \times (155 + 42) = 64.025$ RMB
- (7) The total cost each ram is: $50.05 + 64.025 = 114.075$ RMB**

2. Profit generated by each ram after adding choline

- (1) The increased kidding rate is calculated as $171.4\% - 135.7\% = 35.7\%$
- (2) The average minimum price for the newly born lamb (regardless of male and female) is 600 RMB
- (3) Each ram is matched with 10 ewes at a time using artificial insemination technology
- (4) The increase value of each ram fed with choline is: $35.7\% \times 600 \times 10 = 2142.0$ RMB**

Therefore, adding 0.5% choline is capable of significantly improving the economic benefits of goat farming.