Research Article

DNA Methylation Profile Associated with Fertility Trait in Goat Using Whole-Genome Bisulfite Sequencing

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Abstract | This work designed to investigate the epigenetic regulation of ovulation rate through DNA methylation profile using WGBS. The whole genome DNA was extracted from ovaries of Zaraibi goats belonging to two different fertility groups: high (HFG) and low (LFG) fertility groups. The extracted DNA was subjected to WGS after treatment with bisulfite. The findings declared that a small difference in the DNA methylation levels is present among high and low fertility groups. The methylated C frequencies in contexts: CG, CHG and CHH were 89.89%, 2.39%, 7.72% and 90.19, 2.34%, 7.47% in high and low fertility groups, respectively. This finding showed that the level of methylated C in CG context is in directionally opposite with fertility trait where this level is lower in HFG than that in LFG. In contrast, the levels of methylated C in contexts CHG and CHH are higher in HFG than those in LFG groups. Despite this small difference in the methylation levels, there are many DMR and DMG were identified in the two groups. One-hundred and seventy fertility-related genes with different frequency in methylation levels were selected for functional enrichment analysis and the results declared the strong relation between methylation patterns of DMGs and fertility trait. It is concluded that DNA methylation patterns of Zaraibi goat ovaries may be responsible for difference in ovulation rate trait between high and low fertility groups through their important roles in folliculogenesis and oocyte ovulation rate. Also, this study declared the opposite association between the methylation levels and expressions of differentially methylated genes which are related to fertility phenomena in goats.

Keywords | DNA methylation, Ovulation rate, Litter size, WGBS, Goat.

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INTRODUCTION

DNA methylation is explained as attachment of methyl group to DNA especially to the carbon atom of cytosine base C at 5th position (Blow e al., 2016). Also, the addition of the methyl group may be occurred to the adenine nucleotide (Greer et al., 2015). The C methylation in CpG context is the extensively examined one compared to other contexts; CHG or CHH (An et al., 2018). DNA methylation is considered an important epigenetic factor which can transmit through successive generations (Ming et al., 2021) and it has different important physiological and biological activities such as gene expression and cellular differentiation (Vento-Tormo et al., 2016; Adamkova et al., 2017) and can affect the phenotypes characters in domestic animals (Fan et al., 2020).

DNA methylation can be studied using different sequenc-

ing approaches including whole genome bisulfite sequencing (Zhang et al., 2017). The Next-generation sequencing of DNA treated with bisulfite became the most popular approach to investigate the DNA methylation profile for the whole genome (Jang et al., 2017) where it gives complete information about the methylation profile of cytosine nucleotides among the whole genome with high specificity.

As the levels of economic and living standard increasing, more meat food is required. Lamb meat is preferable and palatable for population in Mediterranean's countries including Egypt (Teixeira et al., 2020). The demand for lamb is much more than the real lamb production in Egypt and this demand for lamb is continuously increasing due to the over populations. Goats are considered the important sources for meat and milk, particularly in dry and semidry regions (Abd-Allah et al., 2019).

Fecundity is an important economic trait for production of goat meat, therefore, the increasing of goat fecundity will dramatically increase the economic benefits (Ahlawat et al., 2015). The litter size is an important indicator for fecundity, and it contributes 74-93% for fecundity. The ovulation rate is the direct and most important factor which controls the litter size and fecundity where it is the major contributor for goat fecundity. In order to breed the higher fecundity goat, it is highly important to investigate the goat ovulation rate trait (Lai et al., 2016), so the present work aimed to investigate the DNA methylation pattern of Zaraibi goat ovaries and its association with fertility trait using the sequencing of bisulfite-treated DNA.

MATERIALS AND METHODS

Sampling

Two groups of Zaraibi goats were used in this study; high fertility group (HFG; goats had 3/litter) and low fertility group (LFG; goats had 1/litter) in the four previous generations. Three animals from each group were sacrificed under the normal conditions (so, it is not needing any ethic permission) and the ovaries were collected for the analysis of DNA methylation.

Whole-genome bisulfite sequencing

WGBS was carried out according to procedures described by Frattini et al. (2017) and An et al. (2018). Genomic DNA was extracted from the collected ovaries of two fertility groups. The genomic DNA was fragmented to 200-300bp and treated with bisulfite using DNA Methylation Kit. After treatment, PCR amplification of DNA was done to constitute DNA library. The sequencing of samples was done using Illumina HiSeq 2500 platform. After sequencing and filtration, the clean reads were recorded and compared to goat reference genome (Dai et al., 2010).

Methylation level analysis

The methylation level analysis was done according to procedures described by Frattini et al. (2017) and An et al. (2018). The clean reads were compared with goat reference genome (Kasper et al., 2012) and the methylation level was recorded in the complete genome.

Analysis of differentially methylated regions (DMR)

The DMRs were determined using methylKit software. Multiple samples data were analyzed together. Logistic regression model was used to analyze the DMR between groups. The DMR differences were expressed as the mean for each group. The significant different methylation levels between the two tested groups were determined (>10%; q value <0.05).

Functional enrichment analysis of DMGs

The functional enrichment of Differentially Methylated Genes was done using Metascape online (https://metascape.org/gp/index.html#/main/step1) (Zhou et al., 2019).

Quantitative reverse transcriptase PCR

To validate the sequencing results of these DMGs, ten genes of them were selected for the assessment of their mRNAs expression level using qRT-PCR. The reaction volume of 20 µl containing 10 µl SYBR Green master mix, 0.6 µl of 10 µM of both primers (Zhang et al., 2017), 1 µl cDNA and the volume was completed with RNase-Free water. The Data were analyzed using the equation described by Livak and Schittgen (2001). *GAPDH* was used as a House-Keeping gene to normalize the results of gene expressions and the statistically significant was $p<0.05$

RESULTS

DNA methylation data

Whole genome DNA bisulfite sequencing was done for six samples: three from each groups, high (HFG) and low (LFG) fertility groups. Averages for total numbers of sequenced basses and reads number were 177,265,899,051 and 1,173,946,351 for HFG and for LFG 177,437,595,615 and 1,175,083,415, respectively. After trimming process, the total numbers of sequenced bases in HFG and LFG were 153,975,467,691 and 153,874,094,771, respectively and total read›s number were 1,168,585,920 and 1,169,067,815 for HFG and LFG, respectively. The means of Q20% and Q30% for clean and full-length reads were 97.73 and 93.72 for HFG and 97.56 and 93.35 for LFG, respectively (Table 1).

Table 1: DNA methylation data

Group	Sample ID	Mean of total read bases	Mean of total reads	GC (%)	Q20 (%)	Q30 (%)
HFG	Raw data	177,265,899,051	1,173,946,351	22.75	97.48	93.36
	Trimming data	153,975,467,691	1,168,585,920	21.32	97.73	93.72
LFG	Raw data	177,437,595,615	1,175,083,415	22.92	97.30	92.98
	Trimming data	153,874,094,771	1,169,067,815	21.44	97.56	93.35

Table 2: Estimated bisulfite conversion rate by sample

Group	Sample ID	Methylated read level measurements	Unmethylated read level measurements	Estimated bisulfite conversion rate (%)	Mean
HFG	H1	37,005	19,311,931	99.81	99.80
	H2	65,234	30,899,149	99.79	
	H3	58,886	28,695,620	99.80	
LFG	L1	67,995	33,267,371	99.80	99.80
	L2	73,083	35,374,241	99.79	
	L3	45,606	23,503,901	99.81	

Table 3: Methylation ratio of each cytosine in CG, CHG and CHH contexts

Group	Contexts	Total coverage C	Methylated coverage C	Methyl%
HFG	CG	713,802,917	531,249,514	74.43%
	CHG	3,600,679,228	14,079,061	0.39%
	CHH	12,071,376,457	45,637,435	0.38%
LFG	CG	696,437,619	513,622,677	73.75%
	CHG	3,477,494,063	13,308,854	0.38%
	CHH	11,592,085,582	42,589,326	0.37%

Bisulfite conversion rate

Bisulfite conversion rate was calculated at CG, CHG, CHH sites within the lambda genome (Table 2). The bisulfite conversion rates were estimated and ranged from 99.79% to 99.81% among samples using lambda phage DNA as a spike-in control.

Methylation level

The results declared that there is a small difference in the DNA methylation profiles between the two groups, where the frequency of methylated C to total C in context CG is 74.43% for HFG and 73.75% for LFG. The frequency of methylated C to total C in context CHG is 0.39% for HFG and 0.38% for LFG and the frequency of methylated C to total C in context CHH is 0.38% for HFG and 0.37% for LFG (Table 3).

The distribution ratios of methylated C in the three contexts: CG, CHG and CHH were 89.89%, 2.39%, 7.72% and 90.19, 2.34%, 7.47% in high and low fertility groups, respectively (Figure 1). This finding showed that the level of methylated C in CG context is in directionally opposite with fertility trait where this level is lower in HFG compared with that in LFG. In contrast, the levels of methylated C in contexts CHG and CHH (where H = A, C or T) are high in HFG compared with those in LFG groups

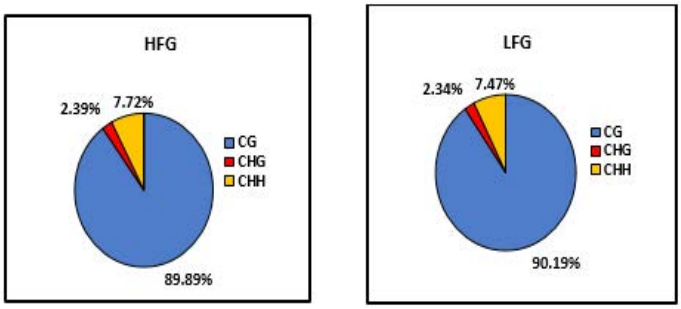

Figure 1: Pie charts of the distribution ratios of methylated C in the three contexts

Identification of differentially methylated region (DMR)

The differentially methylated regions (DMR) with different methylation levels in the two fertility groups (HFG and LFG) were identified. Then, differentially methylated region-related genes (DMG) were determined; where DMR exists within the gene and its upstream 2 kb range, it is considered as a DMR-related gene. Totally, 4640 DMRs were observed, and their analysis showed that most of the

Table 4: List of Differentially Methylated Genes (DMGs)

No.	Gene
1	SOD1
2	APP
3	EPHA3
4	ARL6
5	GSK3B
6	CASR
7	DLG1
8	OPA1
9	TP63
10	PEX5L
11	SMC4
12	EPHB1
13	FAM3B
14	EPHB2
15	CUL3
16	EPHA4
17	BMPR2
18	SGO2
19	STAT1
20	CLASP1
21	GLI2
22	ZEB2
23	ACVR1
24	SLC4A10
25	GRB14
26	STK39
27	LRP2
28	MYO3B
29	SP3
30	ITGA4
31	LIMS2
32	CYFIP1
33	SCLY
34	MACF1
35	TUT4
36	NDC1
37	DAB1
38	NFIA
39	ROR1
40	RPE65
41	PTGER3
42	MSH4
43	PRKACB
44	TGFBR3
45	GFI1

46	STXBP3
47	SORT1
48	MAGI3
49	TBX15
50	IL6R
51	SHC1
52	ASH1L
53	NUF2
54	LMX1A
55	ILDR2
56	MAEL
57	HIPK2
58	AKR1B1
59	GLI3
60	PDE1C
61	BMPER
62	FOXP2
63	MET
64	ASZ1
65	CFTR
66	HGF
67	PCLO
68	HDAC9
69	ICA1
70	AKAP9
71	CDK14
72	GRB10
73	COBL
74	LGR5
75	PTPRQ_
76	KITLG
77	SP1
78	PFKM
79	ARID2
80	NELL2
81	LRIG3
82	NTN4
83	CRY1
84	TM7SF3
85	LRP6
86	ETV6
87	LTBR
88	NTF3
89	TULP3
90	WNK1
91	MEI1

92	PDE5A
93	ENPEP
94	CASP6
95	LEF1
96	DKK2
97	TET2
98	SLC9B2
99	PKD2
100	SLIT2
101	RBPJ
102	PDS5A
103	GRXCR1
104	EPHA5
105	AMTN
106	PPEF2
107	FRAS1
108	PROM1
109	CPLX1
110	MEF2C
111	MSH3
112	FGF1
113	KLHL3
114	HSD17B4
115	CARM1
116	INSR
117	WNT3A
118	NEK1
119	GATA4
120	TEK
121	MLLT3
122	PTPRD
123	RFX3
124	KANK1
125	ALDH1A1
126	ANXA1
127	TLE4
128	RECK
129	PAX5
130	TGFBR1
131	NTRK2
132	PTCH1
133	WNK2
134	ROR2
135	FKTN
136	TNC
137	TPO

138	MYO6
139	MEI4
140	RSPO3
141	MCM9
142	RFX6
143	SLC16A10
144	FOXO3
145	SOBP
146	SIM1
147	UFL1
148	EPHA7
149	MAP3K7
150	CYB5R4
151	EYA4
152	AHI1
153	MAP7
154	MAP3K5
155	EPM2A
156	GRM1
157	SASH1
158	LATS1
159	ESR1
160	OPRM1
161	TSHR
162	MLH3
163	PGF
164	MAP3K9
165	ESR2
166	DACT1
167	ARID4A
168	SLC12A1
169	FGF7
170	MYO5A
171	ALDH1A2
172	MYO1E
173	APH1B
174	CSNK1G1
175	MAPKBP1
176	EXD1
177	SPRED1
178	SCG5
179	FMN1
180	REC114
181	MAP2K5
182	SMAD6
183	MERTK

184	MAP4K4
185	M1AP
186	ALMS1
187	VAX2
188	XDH
189	BIRC6
190	STRN
191	SOS1
192	PRKCE
193	EPAS1
194	RHOQ
195	FSHR
196	WDPCP
197	RAB1A
198	ALK
199	IFT172
200	RAB10
201	NCOA1
202	APOB
203	PRRX2
204	ABL1
205	RAP2A
206	GPC5
207	SCEL
208	UCHL3
209	PIBF1
110	DACH1
211	FGF9
212	ATP8A2
213	FLT1
214	HSPH1
215	RXFP2
216	FOXO1
217	HTR2A
218	CPB2
219	PLCB1
220	JAG1
221	PRKCQ
222	GATA3
223	MASTL
224	NRP1
225	MYO3A
226	ITGA8
227	ZEB1
228	MCM8
229	TRIB3

230	SLA2
231	STK4
232	SULF2
233	KCNB1
234	PTGIS
235	MMP16
236	RIPK2
237	NBN
238	TMEM64
239	STK3
240	GRHL2
241	RRM2B
242	UBR5
243	RIMS2
244	RSPO2
245	SYBU
246	SLC30A8
247	EXT1
248	EYA1
249	CYP7B1
250	CHD7
251	OPRK1
252	ADCY8
253	LRRC6
254	AGO2
255	NCAPD3
256	CRY2
257	ALX4
258	CD44
259	HIPK3
260	FSHB
261	KIF18A
262	LGR4
263	SYT9
264	USP47
265	DKK3
266	TEAD1
267	PDE3B
268	SOX6
269	SORL1
270	DRD2
271	SIK2
272	ATM
273	PIWIL4
274	AMOTL1
275	PGR

276	YAP1
277	MMP8
278	MARK1
279	EXO1
280	PRRX1
281	MTOR
282	NPHP4
283	AJAP1
284	TNN
285	ABL2
286	TDRD5
287	PTGS2
288	PLA2G4A
289	PROX1
290	UTP25
291	ASPM
292	MORC2
293	ZNRF3
294	HNF1A
295	OAS1
296	NOS1
297	TAOK3
298	NCOR2
299	SCARB1
300	PDGFC
301	JADE1
302	CLGN
303	MND1
304	FGB
305	CPE
306	NUP93
307	DPEP3
308	CDH3
309	CDH1
310	SPINT2
311	LTBP4
312	PLAUR
313	BCL3
314	TRPM4
315	TEX14
316	HNF1B
317	NOS2
318	NLK
319	ABR
320	RPH3AL
321	GPS2

322	NDEL1
323	SPAG9
324	HOXB2
325	BRCA1
326	NBR1
327	SOST
328	WNT3
329	MAP3K3
330	ERN1
331	DDX5
332	MAP2K6
333	PRKCA
334	PIK3R1
335	MAP3K1
336	DDX4
337	ITGA1
338	FGF10
339	GHR
340	DAB2
341	NIPBL
342	PRLR
343	LRRK1
344	IGF1R
345	NTRK3
346	IREB2
347	PML
348	BCL11B
349	EGFR
350	NEK10
351	TGFBR2
352	MLH1
353	MYRIP
354	VHL
355	ITPR1
356	MITF
357	LRIG1
358	APPL1
359	HESX1
360	PBRM1
361	LIMD1
362	ATP2B2
363	ATG7
364	PPARG
365	NR2C2
366	BMP6
367	EDN1

368	PKHD1
369	CLIC5
370	RUNX2
371	PTK7
372	DAAM2
373	MAPK14
374	LEMD2
375	GFRAL
376	TSHZ1
377	CDH2
378	TAF4B
379	MIB1
380	GREB1L
381	LPIN23
382	RAB31
383	PTPN2
384	ALPK2
385	BCL2
386	PDPK1
387	ABAT
388	MRTFB
389	MARF1
390	XYLT1
391	PALB2
392	ERN2
393	CUX1
394	SUN1
395	HTRA1
396	FGFR2
397	GRK5
398	EIF3A
399	PNLIP
400	TDRD1
401	TCF7L2
402	GPAM
403	OGA
404	BTRC
405	PLCE1
406	TNKS2
407	RARB
408	KAT6A
409	SFRP1
410	MTNR1A
411	TLR3
412	ING2
413	MCU

414	PSAP
415	SGPL1
416	NID1
417	MAP3K21
418	NOX4
419	GAS2
420	ATRX
421	EDA
422	ACE2
423	GPC3
424	HTR2C
425	AMOT
426	NRK
427	CASK
428	SYTL4
429	NOX1
430	IFT57
431	ADCY5
432	PTX3
433	RYK
434	ARID1A
435	BOLL
436	NR4A2
437	ACVR1C
438	DHRS9
439	ITGA6
440	HOXD3
441	ITGAV
442	KCNQ4
443	HSPB11
444	LEPROT
445	EN2
446	NOD1
447	WNT2
448	HBP1
449	PIK3CG
450	ATP2B1
451	SOCS2
452	IRAK3
453	IGF1
454	APPL2
455	MYH9
456	PDE6H
457	DUSP16
458	PLA2G6
459	DMC1

460	ANXA5
461	TLR6
462	NMU
463	EREG
464	HELQ
465	CPEB2
466	TNIP1
467	RGS14
468	CSF2
469	MAPK9
470	BLK
471	KLF9
472	TLE1
473	DOK2
474	NPM2
475	ASPN
476	SYK
477	GADD45G
478	PHIP
479	HEY2
480	HDAC2
481	SGK1
482	MAP3K4
483	DIO2
484	PKM
485	MAP2K1
486	PAX8
487	ACTR2
488	TNFSF11
489	BMP2
490	STK35
491	TAF4
492	BMP7
493	SOGA1
494	PTPN1
495	ZFAT
496	TRAF6
497	CAT
498	ILK
499	TGFB2
500	AKT3
501	PRKCZ
502	NEK2
503	FGF2
504	GAB1
505	CYLD

506	NOD2
507	DVL2
508	MAP2K4
509	ITGA3
510	DLX3
511	SOCS7
512	STAT5B
513	RPTOR
514	IRX1
515	PAX9
516	DICER1
517	PLCD1
518	CTNNB1
519	TMF1
520	SCAP
521	UBR2
522	GRM4
523	BMP5
524	GALR1
525	TAOK2
526	TRIM72
527	ARL3
528	HELLS
529	PLAT
530	FGFR1
531	MTNR1B
532	ANO1
533	BMP15
534	EPHA4
535	BMPR2
536	SGO2

Table 5: List of fertility-associated DMGs

No.	Gene
1	LGR4
2	ROR1
3	SPATA22
4	CSF1R
5	YTHDC2
6	ACVRI
7	ASH1L
8	ELK4
9	PLA2G4A
10	VMP1
11	LAMB1
12	DPEP3
13	DVL2

14	PDE3A
15	CTNNB1
16	MAEL
17	CPE
18	GATA3
19	STRN
20	ENPP1
21	HPGD
22	DAB1
23	IL15
24	PRKCA
25	SYDE1
26	DMRT1
27	HUS1
28	GPNMB
29	LRRK1
30	SPO11
31	MERTK
32	BRD7
33	FERMT2
34	VHL
35	RXFP1
36	MSX2
37	PTGS2
38	PDE5A
39	MESD
40	PTPRT
41	PAX3
42	CCNE2
43	CSNK2A1
44	PTPRC
45	RRM2B
46	RECK
47	TPH1
48	GRB10
49	LRRK2
50	STAT5B
51	PGR
52	MRE11
53	FRZB
54	CD44
55	LRRFIP2
56	TNKS2
57	TOP2B
58	TRIM72
59	AHSG

60	CDO1
61	BACHI
62	DACT1
63	IL7R
64	A1CF
65	DCDC2
66	ABCG2
67	RAD54L
68	SCEL
69	BRDT
70	MSH5
71	PAK1IP1
72	DYRK3
73	TLE4
74	ASZ1
75	CAV1
76	SMC4
77	TDGF1
78	AURKC
79	CDH2
80	MARK1
81	ANKRD1
82	SENP2
83	mTOR
84	NXN
85	KANK1
86	TIAM1
87	SIAH1
88	ARL6
89	TUT7
90	TNIK
91	ATP2B2
92	PRKAA1
93	PTPRD
94	XDH
95	ROBO1
96	TRPM4
97	INVS
98	FZD6
99	RNF213
100	FERMT1
101	YAP1
102	PRKCB
103	IL12B
104	CCDC88C
105	BTRC

106	M1AP
107	ATM
108	ELP2
109	TNN
110	PRKCQ
111	PMAIP1
112	NPHP3
113	TMEM64
114	RAD1
115	ESPL1
116	FANCM
117	USP34
118	RRN3
119	LRP6
120	EIF2AK3
121	DAAM2
122	GSK3B
123	SOD1
124	MCM8
125	CDIPT
126	ACTR2
127	PIAS1
128	PTCH1
129	PTPN2
130	ALMS1
131	WAPL
132	ATRX
133	SGO2
134	NEUROD1
135	MEIOB
136	SUN1
137	SPRED2
138	JAK1
139	PTX3
140	AKR1B1
141	MLH1
142	DDX5
143	SCYL1
144	TGFBR2
145	PTGIS
146	SPRED1
147	PRKCZ
148	STK3
149	APPL2
150	UBR2
151	PTPRO

152	ASPM
153	PSMD7
154	MLH3
155	STAT1
156	BICC1
157	STK3
158	PRDM14
159	KDM1A
160	RBMS3
161	NPHP4
162	SFRP4
163	TNKS
164	RXFP2
165	CCAR2
166	AMOTL1
167	PRMT6
168	SMC2
169	CDK14
170	CRLF2

DMRs (4615 DMRs) were found in CG context whereas little DMRs were found in CGH (15 DMRs) and CHH (10 DMRs) contexts. The quantity of hypermethylation in CG islands was determined in both HFG and LFG (Figure 2). For HFG, about 65.56% of hypermethylation CGI was present in distal intergenic regions followed by exons and interons with 16.20% and 12.40% of hypermethylation CGI, respectively. The fewer hypermethylation CGI were distributed in promoters (4.12%), 3›UTR (1.20%) and 5›UTR (0.52%). With no significant difference with HFG, the hypermethylation CGI were distributed in LFG as follows: 65.45% in distal intergenic regions, 15.85% in exons, 12.90 in introns, 4.01 in promoter, 1.30 in 3›UTR and 0.49% in 3›UTR.

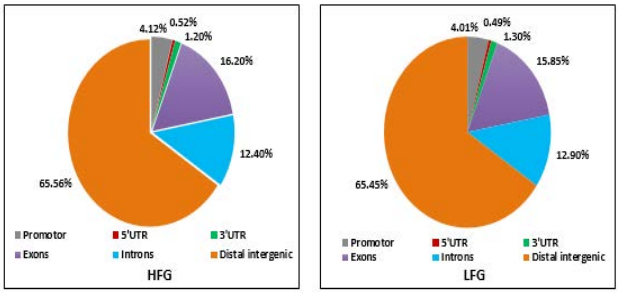

Figure 2: Pie charts of the distribution ratios of CGI in both high and fertility groups

Functional enrichment of methylated genes (DMG)

The enrichment analysis declared that some of DMGs are involved in the reproductive system development and many functional pathways which are related to follicle development and other important functional pathways responsible on regulation of growth, hormone production and development. The data declared that DMGs in goat ovaries are associated with the fertility phenomena in both fertility groups. Five-hundred and thirty-six DMGs (Table 4) from these DMRs were further analyzed in both fertility groups into HM (hyper-DMGs in HFG group) and LM (hyper-DMGs in LFG group where there were 1505 and 1135, respectively. The protein-coding regions (CDS) in these DMGs were determined (Figure 3) and enrichment analysis of protein coding regions declared their involvement in different important pathways (Figure 4).

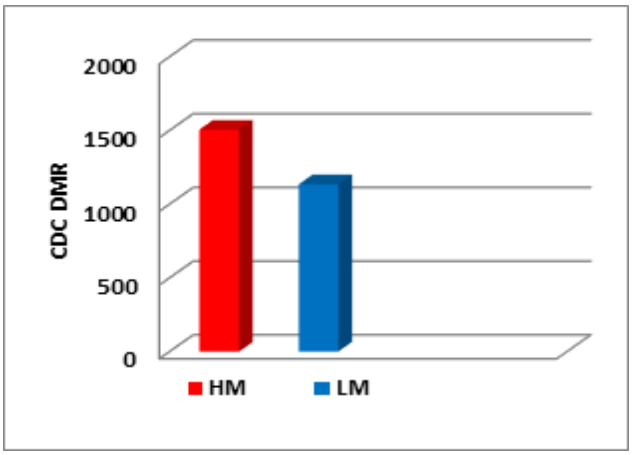

Figure 3: Distribution of protein-coding region (CDS) into HM and LM

One-hundred and seventy fertility-related genes with different methylation levels were selected for further functional enrichment analysis and the results declared that these genes with their methylation patterns are responsible for the difference in the fertility phenomena between high and low fertility groups (Table 5).

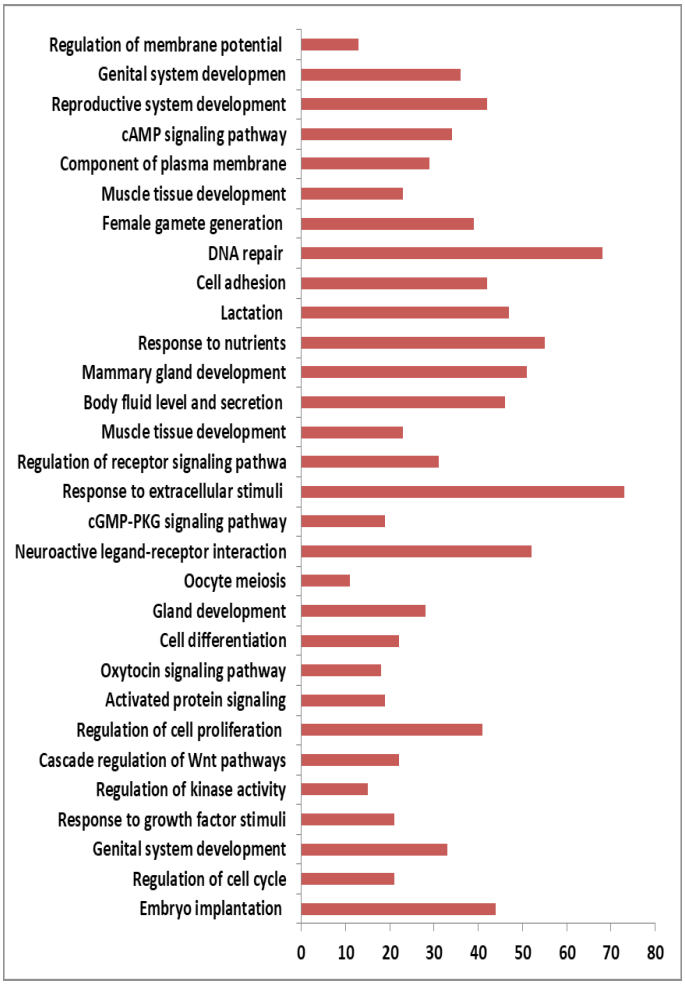

Figure 4: The enrichment analysis of protein coding regions (p<0.05)

Gene expression of selected DMGs by qRT-PCR

Ten DMGs were randomly selected to assess their gene expression for validation the association between their methylation patterns and the fertility trait. Data were represented as the fold change in target gene expression normalized to *GAPDH* gene in HFG relative to LFG. The mean cycle threshold (Ct) values of triplicate samples for each gene were calculated and used in data analysis. The results declared that the expressions of five tested DMGs: SCYL1, mTOR, ABCG2, STK3 and PSMD7 were higher in HFG than those in LFG, with significantly level (p<0.01) for SCYL1, mTOR, ABCG2 genes whereas the expressions of STK3 and PSMD7 genes were insignificant increasing (Figure 5). The expressions of other five genes were lower in HFG than those in LFG: GPNMB, ELK4, BACH1 at significant level p<0.01, CDIPT with significant level p<0.05 and ACVR1 with insignificant value. Generally, the qRT-PCR results were concordance with the sequencing data and confirming the reliability of sequencing data.

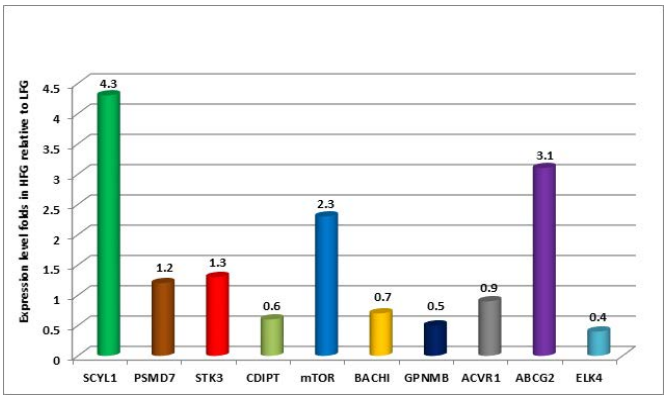

Figure 5: The expression level of DMGs as determined by qRT-PCR in HFG relative to LFG

DISCUSSION

The domestic goat is an important small ruminant which distribute all over the world, especially in desert and semi-desert regions where goat is easier to breed with less expensive than other large livestock (Nowier et al., 2019). The improvement of goat productivity has a respectable role for covering the gap between over-growing populations and adequacy of milk and meat supplies (Miller and Lu, 2019). Production improvements of farm animals can be achieved by using new genetic technology for better selection of heritable traits. Selection has done depending on economically important productivity traits such as disease resistance, fertility performance as well as meat and milk production (Gutierrez-Reinoso et al., 2021).

The litter size represents one phenomenon associated with fertility performance in goats and it is related to the healthy reproductive organs and high ovulation rate (Assan et al., 2021). DNA methylation is an epigenetic factor which has an important role in gene expression regulation. In farm animals, DNA methylation regulates sexual, ovarian maturation and ovulation rate (Breton-Larrivée et al., 2019). Genome-wide bisulfite sequencing was used to explore the methylation profiles of genes which are related to complex trait such as litter size or ovulation rate trait (Lai et al., 2016).

The genome-wide methylation levels were assessed in different farm animals which discussed the association between DNA methylation profiles, gene expression and phenotype characters related to production and reproduction traits (Hao et al., 2016; An et al., 2018; Fan et al.,

2020). Recently, the DNA methylation profiles in sheep ovaries and their associations with different parameters of fertility trait were described (Barboni, et al., 2011; Russo et al., 2013; Zhang et al., 2017). Regarding to goat, An et al. (2018) studied the change in the whole-genome methylation profiles between oestrous and dioestrous stages. The present study was focused on the assessment of methylation pattern in the whole genome goat ovaries and its relationship with the difference in litter size and ovulation rate traits. Our results showed a slightly non-significant increase in DNA methylation level in goat ovaries in high fertility group over than that in low fertility group despite the detection of many DMRs and DMGs among the two fertility groups. The similar finding was reported by Kang et al. (2022) who studied the genome-wide DNA methylation pattern in goat ovaries of Chinese Ji›ning grey goats with different litter size groups.

In the present study, most of methylated C was present in CG context and this result agrees with other reports in different species including human (Lister et al., 2014), pig (Hao et al., 2016), sheep (Zhang et al., 2017) and Chinese Ji›ning grey goats (Kang et al., 2022). Our finding declared that the major hypermethylation CGI in Zaraibi goat breed was present in distal intergenic regions followed by exons and interons whereas the lowest frequencies of CGI were in 3›UTR and 5›UTR. These proportions of methylated CGI were nearly like those which were reported in Chinese sheep and goat (Zhang et al., 2017; Kang et al., 2022).

One hundred and seventy DFGs were selected for the enrichment analysis and the result showed their role in different biological functions related to fertility. The directionally opposite association between methylation levels of ten selected DMGs and their gene expression was confirmed where SCYL1, mTOR, ABCG2, STK3 and PSMD7 were hypomethylated with highly expression in HFG compared with LFG. On the other hand, GPNMB, ELK4, BACH1, CDIPT and ACVR1 were hypermethylated with decreased expression in HFG than those in LFG. This directionally opposite association between methylation levels of DMGs and their gene expression was also reported by Kang et al. (2022) in goat and by Zhang et al. (2017) in sheep.

CONCLUSION

It is concluded that DNA methylation patterns of Zaraibi goat ovaries may be responsible for the fertility trait in goats through their important roles in folliculogenesis, oocyte ovulation rate and finally the fertility phenomena. Also, this study confirmed the opposite association between the methylation levels and expressions of differentially methylated genes which are related to fertility phenomena in goats.

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CONFLICT OF INTEREST

There is absolutely no conflict of interest between the authors of this manuscript and any other scientists or producers.

FUNDING

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NOVELTY STATEMENT

To our knowledge, this study is the pioneer one for providing a comprehensive analysis of whole-Genome DNA methylation patterns in goat ovaries which helps for understanding the relation between ovarian DNA methylation and Egyptian goat fertility.

AUTHORS CONTRIBUTION

All authors certify that they have participated sufficiently in contributing of the manuscript. Othman E. Othman: Work design, Methodology, Data analysis and Manuscript writing. Lingjiang Min: Work design, Primer providing and Data analysis. Amira M. Nowier: Providing animals and all data associated with their fertility records.

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