*Ann. Anim. Sci., Vol. 15, No. 3 (2015) 641–653* *DOI: 10.1515/aoas-2015-0007*

DE GRUYTER
OPEN

# EFFECT OF ALTITUDE ON SOME MALE FERTILITY RELATED TRAITS IN SAUDI OVINE AND CAPRINE SPECIES

Nabil S. Awad[1,2,3♦], Mohamed M. Soliman[4,5], Alaa A. Mohamed[1,2,6], Ayman M. Sabry[1,2,7], Ahmad F. Shahaby[1,2,8], Adel E. El-Tarras[1,2,9]

[1]High Altitude Research Center (HARC), Taif University, Saudi Arabia
[2]Biotechnology and Genetic Engineering Unit, Scientific Research Deanship, Taif University, Saudi Arabia
[3]Department of Genetics, Faculty of Agriculture and Natural Resources, Aswan University, Aswan, Egypt
[4]Department of Biochemistry, Faculty of Veterinary Medicine, Benha University, Egypt
[5]Medical Laboratory Department, Faculty of Applied Medical Sciences, Turabah, Taif University, Saudi Arabia
[6]Department of Animal Reproduction and AI, Veterinary Research Division, National Research Center, Dokki, Giza, Egypt
[7]Department of Cell Biology, National Research Center, Dokki, Giza, Egypt
[8]Cairo University, College of Agriculture, Cairo, Egypt,
[9]Genetics Department, Faculty of Agriculture, Cairo University, Cairo, Egypt
♦Corresponding author: nabilfaris151@yahoo.com

**Abstract**

**This study aimed to evaluate the effect of altitude on some male fertility related traits in Saudi sheep and goats. Testicular tissues were collected from a slaughterhouse in Taif governorate (1800 m above sea level) and Makkah governorate (sea level). Sperm characteristics (1 – individual motility, 2 – percent live sperm 3 – percent abnormal sperm) were examined. Semi-quantitative RT-PCR assay was used to evaluate the expression of IGF-II, StAR, LDLr and CYP11A genes. No significant effect of altitude on tested sperm parameters was revealed. Expression of IGF-II gene in both sheep and goats was significantly (P<0.05) higher at sea level compared to high altitude. A similar effect of altitude on StAR gene expression was only observed in goats, while in sheep the level of effect did not reach the significance threshold. Moreover, LDLr gene expression was significantly (P<0.05) higher for both sheep and goats at high altitude than at sea level. The CYP11A gene expression was significantly (P<0.05) higher in high altitude sheep than in those raised at sea level, while the opposite trend was observed for goats. In conclusion, high altitude had an effect on the expression of some studied male fertility related genes, but sperm parameters were not significantly affected.**

**Key words: high altitude, fertility, sheep, goat, sperm parameters, gene expression**

Elevation above sea level reduces partial pressure of oxygen ($PO_2$), which is known as hypobaric hypoxia (HH) (Reeves and Weil, 2001). During hypobaric hypoxia, multiple physiological processes are affected (Bustos-Obregon et al., 2006) such as reduction of fecundity in human populations resident at high altitude (Verrati and Di Giulio, 2012; Bomhard and Gelbke, 2013). Exposure to HH for more than 60 days resulted in morphological injury in the seminiferous tissue, associated with energetic and metabolic alterations in spermatogenic cells (Farias et al., 2005). Exposure to chronic simulated hypobaric hypoxia (CSHH) induced damage in the seminiferous epithelium, a decrease of lipoperoxidation in spermatozoa and testicular tissue, and damage to the testicular and sperm morphology (Bustos-Obregon et al., 2006). Velickovic and Stefanovic (2014) stated that, in healthy men, chronic hypoxia induces a state of reversible oligozoospermia. A reduced sperm count can be related to the increase in germ cell apoptosis promoted by this hypoxic condition.

Spermatogenesis is affected by high altitude exposure, particularly the onset of mitosis and spermiation in rat (Gasco et al., 2003) and human (Verrati and Di Giulio, 2012). It has been shown that hypoxia exposure induces a partially reversible decrease in semen volume, sperm count and sperm motility (Saxena, 1995; Verratti et al., 2008) and an increase in abnormal or immature spermatozoa in human (Verratti et al., 2008). Also, semen analyses of the members of the Masherbrum expedition (7821 m above sea level) showed a reversible sperm count decrease, an increase in abnormal shaped sperm but no change in semen volume (Okumura et al., 2003). Damage to mature spermatozoa after hypoxia exposure was also described by Bustos-Obregon and Olivares (1982). Histological examination of rat testis after hypoxia showed changes in testicular morphology, loss of spermatogenic cells at all stages of the spermatogenic cycle, degeneration of the germinal epithelium and spermatogenic arrest, degeneration and sloughing of spermatogenic cells in occasional tubules and differences in the volume of the testis occupied by Leydig cells (Gosney, 1984; Farias et al., 2005). These changes are associated with an increase in interstitial space and in testicular mass, a decrease in height of the seminiferous epithelium, depletion of cellular elements and vacuolization in epithelial cells and folding of the basal membrane (Farias et al., 2005).

High attitude exposure stressors such as cold and hypoxia are known to have profound effects on genome-wide pattern of gene expression (Cheviron et al., 2008; Cheviron and Brumfield, 2012). The exposure to high altitude hypoxia affected the expression of both IGF-I and IGF-II in female sheep (Parraguez et al., 2013). Adrenal mRNAs for CYP17, CYP11A1, and the ACTH receptor were significantly reduced in high altitude sheep compared with levels shown in controls (Myers et al., 2005).

Several genes are controlling the male fertility process. Insulin-like growth factor II (IGF-II), cholesterol, Steroidogenic Acute Regulatory (StAR), low density lipoprotein receptor (LDLr) and Cytochrome P450 (CYP11A) genes are involved in many steps of spermatogenesis and testosterone production. IGF-II gene expression has been localized by *in situ* hybridization in testis and epididymis and found to be increased by treatment with FSH and LH (Dombrowicz et al., 1992). IGF-II is produced locally in the testis and found to play an important role in the local regulation of testicular function (Peters et al., 2003).

Male fertility depends mainly on *de novo* synthesis of androgens from cholesterol. Steroidogenic Acute Regulatory (StAR) gene encoding protein transfers cholesterol from the outer membrane to the inner mitochondrial membrane (Luo et al., 2011). StAR is considered to be the limiting step in testosterone biosynthesis and reduced StAR is always found in testicular dysfunction (Wang et al., 2010). Low density lipoprotein receptor (LDLr) gene plays an important role in male fertility by mediating the endocytosis of LDL and other cholesterol-carrying particles. Impairment of LDL receptor activity results in the accumulation of LDL in the circulation and decreased level inside cell which in turn decreases testosterone biosynthesis from cholesterol (Fagundes et al., 2005).

Cytochrome P450, a gene encoding cholesterol side-chain cleavage enzyme (CYP11A) is affected by steroidogenesis. CYP11A is a mitochondrial enzyme that catalyzes conversion of cholesterol to pregnenolone. This is the first reaction in the process of steroidogenesis in all mammalian tissues that specialize in the production of various steroid hormones (Hanukoglu, 1992).

Most findings about altitude effects have been examined in humans and experimental animals, less is known about small ruminants. Therefore, the present work aimed to study the effect of altitude on some sperm characteristics (motility, live percentage and abnormalities percentage) and expression of testicular genes (IGF-II, StAR, LDLr and CYP11A) in Saudi ovine and caprines.

## Material and methods

This study was carried out in 2 regions (Taif as a high altitude region and Makkah as a sea level region). Testicular tissues from Sawakni sheep breed and Harri goat breed were collected from slaughterhouses in both areas under completely sterile conditions. Samples were collected from both goat and sheep breeds of the same mature age. Testicular samples (approximately 100 mg per sample) were taken immediately after slaughter in TriZol for RNA extraction and gene expression analysis and stored at –80ºC. The rest of testicular sample were transferred to the laboratory for sperm analysis.

### Sperm analysis

The epididymal sperm was collected according to Blash et al. (2000) with some modification. The testes were removed from the scrotal sac within 5 to 10 minutes, placed in an insulator box and transported to laboratory and processed individually. The parietal tunic was removed leaving the tail of the epididymis exposed. A small lateral incision was made along the tail of the epididymis to open the convoluted tubules and put in petri dish with 3.025 g Tris, 1.7 g citric acid, 1.25 g fructose (TFC) supplemented with 5.5 mg tylosin, 27.5 mg gentamycin, 16.5 mg lincospectin, and 330 mg spectinomycin per 100 ml. Spermatozoa were sedimented by gentle centrifugation at 800 X g (1200 rpm) for 5 min at 30°C and the pellet was washed twice with TFC medium to remove contaminating epididymal plasma. The cells were dispersed

in the same medium and this preparation of spermatozoa was used for the experiments.

Individual sperm motility was assessed by bright field microscopy. Diluted sperm was examined microscopically using adjusted hot stage microscope at 38ºC. Individual sperm motility percent was determined on a subjective scale of 0–100% to the nearest 5% after examining several microscopic fields. The percentage of live and abnormal sperms was assayed by staining smears with eosin-nigrosin (Campbell et al., 1956). A total of 200 sperm cells were examined randomly. Total sperm abnormalities and live percentage were recorded.

**RNA extraction**

For preparation of total RNA, frozen samples were thawed then homogenized using a Polytron 300 D homogenizer (Brinkman Instruments, Westbury, NY). Then 0.3 ml chloroform was added to the homogenate. The mixtures were shaken for 30 seconds followed by centrifugation at 4°C and 12,500 rpm for 15 min. The supernatant aqueous clear layer was transferred to a new set of tubes, and an equal volume of isopropyl alcohol was added to the samples, shaken for 15 seconds and centrifuged at 4°C and 12,500 rpm for 15 min. The RNA pellets were washed with 70% ethanol, briefly dried up and then dissolved in diethylpyrocarbonate (DEPC) water. RNA concentration and purity were determined spectrophotometrically at 260 nm using Bio-RAD-SmartSpec Plus spectrophotometer. The ratio of the 260/280 optical density of all RNA samples was 1.7–1.9 to check the purity of RNA used for RT-PCR analysis.

**cDNA synthesis and semi-quantitative PCR analysis**

For synthesis of cDNA, a mixture of 2 µg total RNA and 0.5 ng oligo dT primer in a total volume of 11 µl sterilized DEPC water was incubated in an Eppendorf® thermal cycler at 65°C for 10 min for denaturation. Then, 4 µl of 5X RT-buffer, 2 µl of 10 mM dNTPs and 100 U Moloney Murine Leukemia Virus (M-MuLV) Reverse Transcriptase (SibEnzyme Ltd. Ak, Novosibirsk, Russia) were added and the total volume was completed up to 20 µl by DEPC water. The mixture was then re-incubated in the thermal cycler at 37°C for 1 h, then at 90°C for 10 min to inactivate the enzyme. Specific primers for examined genes (Table 1) were designed using Oligo-4 computer program and synthesized by Macrogen (Macrogen Company, GAsa-dong, Geumcheon-gu, Korea). PCR was conducted in a final volume of 25 µl consisting of 1 µl cDNA, 1 µl of 10 picomolar (pM) of each primer (forward and reverse), and 12.5 µl PCR master mix (Promega Corporation, Madison, WI) volume was brought up to 25 µl using sterilized, deionized water. PCR was carried out using an Eppendorf® thermal cycler with the cycle sequence at 94°C for 5 minutes per cycle, followed by different cycles according to each gene; each cycle consisted of denaturation at 94°C for one minute, annealing at the specific temperature corresponding to each primer and extension at 72°C for 1 minute with additional final extension at 72°C for 7 minutes. Changes in mRNA level were detected using RT-PCR and calculated as proportion of the target gene amplification product to the amplification product of housekeeping gene 18s mRNA. Names and sequences of the used

specific primers are illustrated in Table 1. PCR products were electrophoresed on 1.5% agarose gel (Bio Basic Inc., Markham Ontario, Canada) stained with ethidium bromide in Tris-Borate-EDTA (TBE) buffer. PCR products were visualized under the UV light and documented using a GeneSnap 4.00 Gene Genius Bio Imaging System (Syngene; Frederick, Maryland, USA). The intensities of the bands were quantified densitometrically using ImageJ software (http://imagej.en.softonic.com/).

Table 1. Primer sequence and PCR conditions of examined genes using RT-PCR analysis in testicular tissues of sheep and goat

| mRNA expression | Forward primer (5‘-3‘) | Reverse primer (5‘-3‘) | PCR cycles and annealing conditions |
|---|---|---|---|
| IGF-2 (277bp) | 5‘-CGTGGCATCGTGGAA-GAGTGT- 3’ | 5-GGTGACTCTTGGC-CTCTCTGA-3’ | 30 cycles, 60°C for 1 min |
| LDLr (337 bp) | 5’- GACGAGGAGAACTGC-GATGT-3’ | 5‘-GGCACTCATAGC-CGATCTTG-3’ | 33 cycles, 60°C 1 min |
| StAR (393bp) | 5-’GGTTCTCAGCTGGAA-GACAC-3’ | 5’-GGTACAGCCGACACT-CACAA-3’ | 33 cycles, 60°C for 1 min |
| CYP11a (304 bp) | 5’-CTCGGCAACTTG-GAATCTGT-3’ | 5’-AGCCATTACCTCCGT-GTTCA-3’ | 35 cycles, 60°C for 1 min |
| Ovine 18S (315 bp) | 5’-CGATGCTCTTAGCT-GAGTGT-3’ | 5-GGAACTACGGTATCT-GA-3’ | 28 cycles, 59°C 1 min |

PCR cycles of respective genes are shown, while temperature and time of denaturation and elongation steps of each PCR cycle were 94°C, 30 sec and 72°C, 60 sec, respectively.

**Statistical analysis**

Results are expressed as means ± standard error (SE). Statistical analysis was done using ANOVA and Fisher’s post hoc test, with $P<0.05$ being considered as statistically significant.

## Results

**Effect of high altitude on sperm characteristics in sheep and goats**

The changes in motility, percentages of live and abnormal sperms were shown in Table 2. As seen in Table 2, sperm motility and live percentage were low in goats and sheep from Taif compared to Makkah area. Abnormal sperm percentages were high in goats and sheep from Taif compared to Makkah area but did not reach the level of significance. The overall results showed that high altitude affected tested sperm parameters without significant effect in both sheep and goats.

Table 2. The sperm characteristics of goats and sheep from Taif and Makkah areas (values are mean ± standard error)

| | Motility (%) | | Live Sperm (%) | | Abnormal Sperm (%) | |
|---|---|---|---|---|---|---|
| | Taif | Makkah | Taif | Makkah | Taif | Makkah |
| Goat | 71.11±2.17 | 73.89±1.82 | 87.44±3.28 | 88.33±1.39 | 14.44±1.09 | 13.33±0.82 |
| Sheep | 75.00 ±1.86 | 77.22±1.21 | 89.44±1.64 | 90.78±1.26 | 10.33±0.78 | 9.00±0.41 |

**Effect of high altitude on IGF-II gene expression in sheep and goats**

The results of IGF-II mRNA expression in sheep and goats from high altitude and sea level were shown in Figure 1. Densitometric analysis (Figure 2) showed that the expression of IGF-II in Makkah was significantly higher ($P<0.05$) than in Taif area in both sheep and goats.

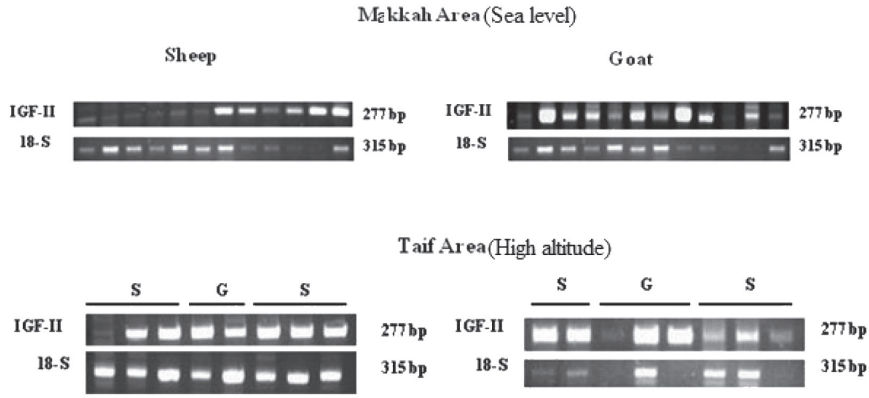


Figure 1. RT-PCR analysis of IGF-II expression in testis of sheep (S) and goats (G) in different altitude areas

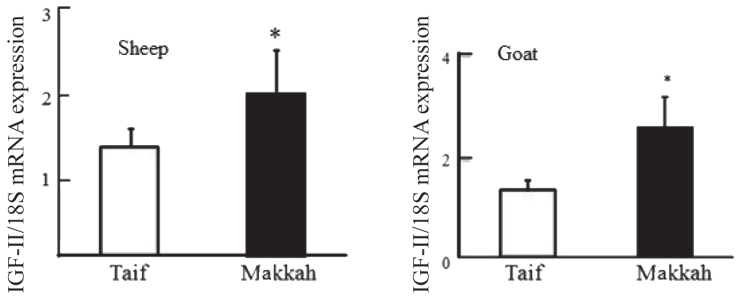


Figure 2. Densitometric analysis of IGF-II expression in testis of sheep at Taif and Makkah area. Values are expressed as means ± standard error. Means are significant at *$P<0.05$

**Effect of high altitude on StAR gene expression in sheep and goats**

The effects of altitude exposure on mRNA level of testicular StAR in sheep and goats were determined by RT-PCR analysis. As shown in Figures 3 and 4, high altitude (Taif area) decreased mRNA level of StAR in testes of both sheep and goats. The effect of high altitude was found to be only significant in goats.

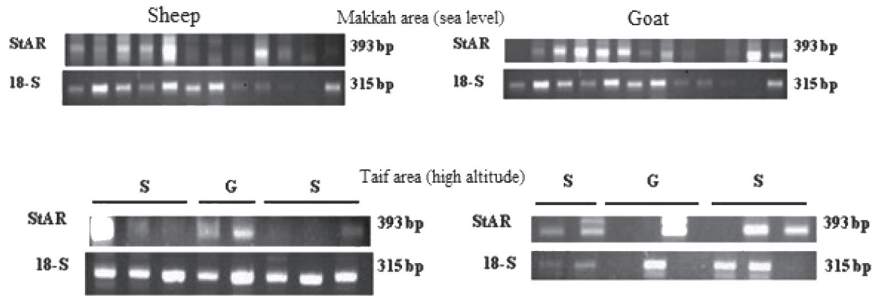


Figure 3. RT-PCR analysis of StAR expression in testes of sheep (S) and goat (G) in different altitude areas

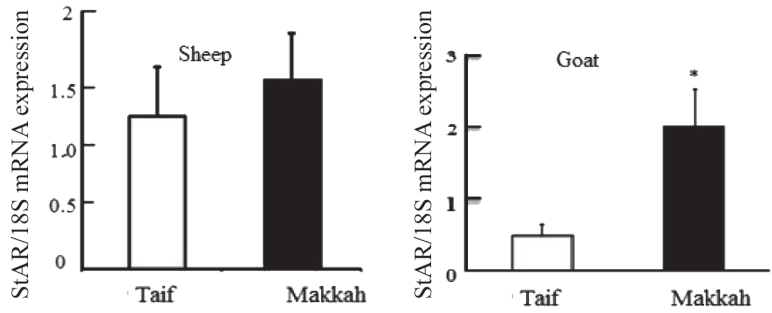


Figure 4. Densitometric analysis of the expression of StAR in testis of sheep in Taif and Makkah area. Values are expressed as means ± standard error. Means are significant at *P<0.05

**Effect of high altitude on LDLr gene expression in sheep and goats**

Patterns of LDLr gene expression are shown in Figure 5. Densitomteric analysis (Figure 6) showed a significant increase in gene expression level at high altitude (Taif) compared to Makkah (sea level) for both species.

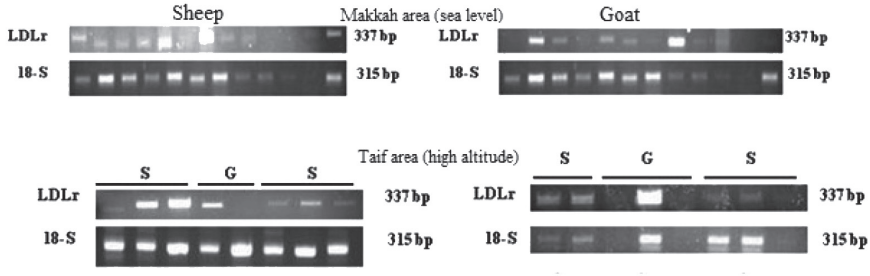


Figure 5. RT-PCR analysis of LDLr expression in testes of sheep (S) and goat (G) in different altitude areas

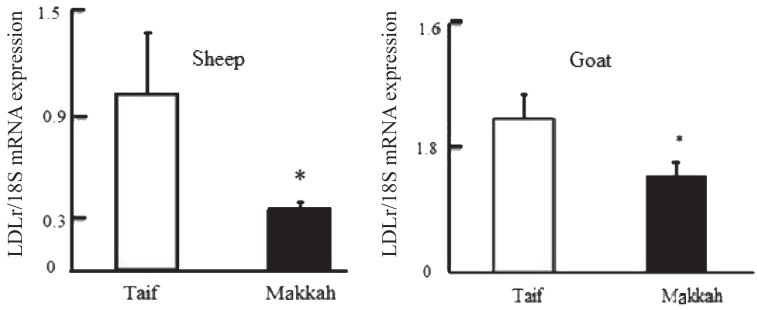


Figure 6. Densitometric analysis of the expression of LDLr in testis of sheep in Taif and Makkah area. Values are expressed as means ± standard error. Means are significant at *P<0.05

**Effect of high altitude on CYP11A gene expression in sheep and goat**

Ambiguous patterns of CYP11A gene expression were detected for both altitude and species (Figures 7 and 8). The gene expression of CYP11A in sheep raised at Taif (high altitude) was found to be significantly higher than that for sheep raised at Makkah (sea level). However, for goats raised at Makkah (sea level) CYP11A gene expression was found to be significantly higher than for goats raised at Taif (high altitude).

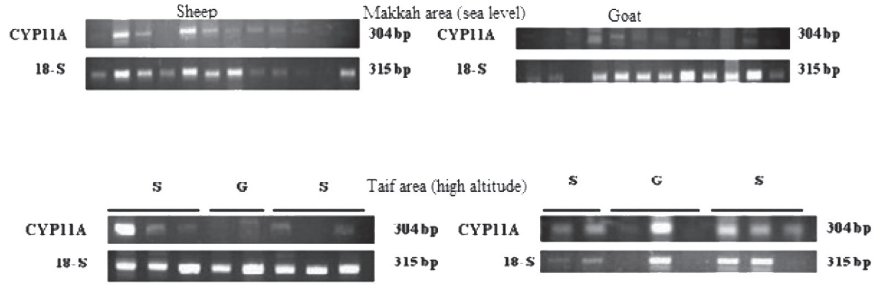


Figure 7. RT-PCR analysis of CYP11A expression in testes of sheep (S) and goat (G) in different altitude areas

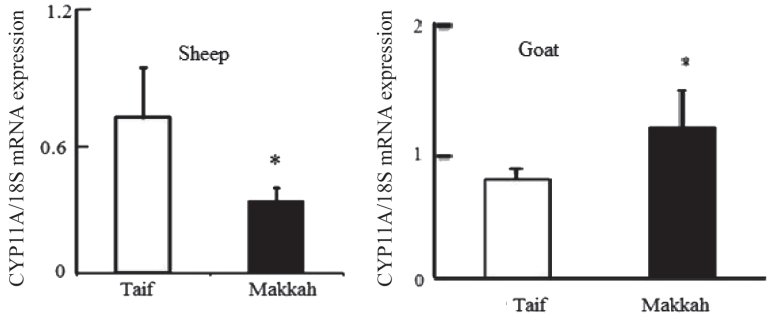


Figure 8. Densitometric analysis of the expression of CYP11A in testis of sheep in Taif and Makkah area. Values are expressed as means ± standard error. Means are significant at *P<0.05

## Discussion

High altitudinal hypobaric hypoxia acts as a stressor that reduces fecundity (Vargas et al., 2011). Low fertility was observed in human populations residing at high altitude (Abelson 1976; Verratti and Di Giulio, 2012).

The obtained results of sperm characteristics in sheep and goats show the absence of significant differences between the two regions: Taif (1800 m above sea level) and Makkah (sea level). Our results are contrary to the reports in men (Okumura et al., 2003), rat (Cikutovic et al., 2009), and mice (Vergas et al., 2011), which confirmed the effect of high altitude on sperm motility, live and abnormality percentages, but are in accordance with those obtained by Garcia-Hjarles (1989) who examined semen volume and sperm motility in healthy men at sea level and at moderate altitude (4300 m). The author found sperm volume and motility to be insignificantly lower at moderate altitude compared to sea level. These insignificant variations may be due to moderate altitude of the Taif region, accompanied by moderate level of hypoxia that in turn moderately affects the studied sperm characters. Two other possible explanations are either the acclimatization process undergone by animals kept in Taif governorate or/and sample size.

High altitude altered the mRNA expression of IGF-II, StAR, LDLr and CYP11A (genes related to stability of fertility). The decrease in percentage of live, motile and abnormal forms of sperms reported in our study is consistent with the decrease in mRNA expression of steroidogenesis related enzymes essential for testosterone biosynthesis and normal sperm production. Moreover, another possible explanation is the decrease in oxygen concentration inside testicular tissues and consequently low anaerobic glycolysis as reported by Abdulmalek et al. (2001).

There are some indirect evidences that the insulin like growth factors axis might be involved in preserving precursor and immature Leydig cell (LC) pools, as well as in the modulation of local steroidogenesis (Ge et al., 2006). As in an IGF-I KO mice model, Baker et al. (1996) found a reduced number of mature LCs, and Wang et al. (2003) reported that IGF-I was a critical factor in the control of adult LC number and LC maturation. Therefore, the presence of IGF-I was required for cell maturation and function. In addition, it has been reported that IGFs increase proliferation and differentiation of rodent LC precursors, as well as the steroidogenic response to LH, in fetal and adult LCs in culture (Wang et al., 2003). Parallel to that well established data, IGFs especially IGF-II plays a role in improvement of male infertility (Nakayama et al., 1999; Colon et al., 2005; Berensztein et al., 2008; Park et al., 2010). Our findings showed that IGF-II gene expression was lower in Taif than Makkah breeds. This result might be due to the elevation above sea level in Taif area. Elevation above sea level negatively affected the secretion of both growth hormone and follicle stimulating hormone (FSH) (Nelson et al., 1975; Çoksevim et al., 2006), where both hormones are the main controller of IGF production (Park et al., 2010).

Our findings revealed down regulation of StAR gene expression in Taif area compared to Makkah area for both sheep and goats. This down regulation of StAR mRNA level at high altitude could be attributed to the direct effect of hypoxia on testicular cells as stated by Zhang et al. (2012, 2013), who reported that the decrease

in StAR expression by the intermittent hypoxia causes a low oxygen tension in the bloodstream leading to damage of cellular organelles, reduction of serum testosterone, abnormal sexual behaviour and marked changes in morphology of the testes. Another possible explanation of the down regulation of StAR gene might be due to the oxidative stress induced by high altitude, since STAR gene expression is found to be sensitive to different stressors such as insecticides (Wang et al., 2010) and aflatoxins (Adedara et al., 2014). On the other hand, contradictory findings were obtained by Bruder et al. (2007) and Vargas et al. (2011). They reported that high expression of StAR gene was higher at induced hypoxia. This contradiction might be ascribed to the differences in organs used in both studies, as their study was carried out on adrenal glands and not on testicular tissues.

A significant increase of LDLr gene expression in Taif compared to Makkah area in both sheep and goats was observed. As LDLr and StAR are considered as key players for influx of cholesterol inside the mitochondria to start testosterone synthesis and production, so the observed increase of LDLr gene expression might be a compensatory mechanism for the decline in StAR gene expression. The results were in agreement with Bruder et al. (2007)**.**

As is known, CYP11A is the enzyme that catalyzes the conversion of cholesterol to pregnenolone. Our results revealed a significant increase in sheep CYP11A gene expression in Taif compared to Makkah area; this escalation in gene expression may be another compensatory mechanism for decrease in StAR gene expression. The increase of CYP11A gene expression in response to different stressors induced by altitude is in agreement with the findings of Adedara et al. (2014), who found an increase in CYP11A gene expression as a response to aflatoxin treatment and explained the increase as a homeostatic adjustment triggered by decreased cholesterol availability. Chung et al. (2011) emphasized that the benzo[*a*]pyrene (insecticide) increases the CYP11A gene expression with marked decrease in androgen secretion. In goat CYP11A gene expression was lower in Taif than Makkah and was not affected by the stress induced by high altitude. Moreover, exposure to cypermethin (insecticide) did not affect the expression of CYP11A. Inconsistency of results of CYP11A gene expression between sheep and goat breeds shows a different response between species to stress induced by high altitude (Wang et al., 2010). Further and thorough investigations are needed to outline the direct *in vitro* effects of altitude on the expression of steroidogenesis related enzymes in ovine and caprine testicular cells.

**Conclusions**

High altitude above sea level affected the expression of studied fertility related genes but not sperm parameters in sheep and goats. Moreover, there was no stereotypic influence of high altitude on expression of the four male reproductive related genes considered in the present study, since the response of the gene expression varied with the altitude and species as well. Due to the importance of reproduction process, the presence of compensatory mechanism (to any possible fluctuation in gene expression) is a biological tool to maintain a certain level of reproduction, such that down regulation in expression of some genes is compensated by up regulation

of another. This mechanism may provide an explanation for the results of the present study.

**References**


Abdulmalek K., Ashur F., Ezer N., Fengchun Y., Magder S., Hussain S.N.A. (2001). Differential expression of Tie-2 receptors and angiopoietins in response to *in vivo* hypoxia in rats. Am. J. Physiol., 281: L582– L590.

Abelson A.E. (1976). Altitude and fertility. Human Biology, 48: 83–92.

Adedara I.A., Nanjappa M.K., Farombi E.O., Akingbemi B.T. (2014). Aflatoxin $B_1$ disrupts the androgen biosynthetic pathway in rat Leydig cells. Food Chem. Toxicol., 65: 252–259.

Baker J., Hardy M.P., Zhou J., Bondy C., Lupu F., Belleu A.R., Efstratiadis A. (1996). Effects of an IGF-I gene null mutation on mouse reproduction. Mol. Endocrinol., 10: 903–918.

Berensztein E.B., Baquedano M.A.S., Pepe C.M., Costanzo M., Saraco N.I., Ponzio R., Rivarola M.A., Belgorosky A. (2008). Role of IGFs and insulin in the human testis during postnatal activation: differentiation of steroidogenic cells. Paediatric Research, 63: 662–666.

Blash S., Melican D., Gavin W. (2000). Cryopreservation of epididymal sperm obtained at necropsy from goats. Theriogenology, 54: 899–905.

Bomhard E.M., Gelbke H.P. (2013). Hypoxaemia affects male reproduction: a case study of how to differentiate between primary and secondary hypoxic testicular toxicity due to chemical exposure. Arch. Toxicol., 87: 1201–1218.

Bruder E.D., Lee J.J., Widmaier E.P., Raff H. (2007). Microarray and real-time PCR analysis of adrenal gland gene expression in the 7-day-old rat: effects of hypoxia from birth. Physiol. Genomics, 29: 193–200.

Bustos-Obregón E., Olivares A. (1982). Efecto de la hipoxia en la reproducción demamíferos. El hombre y los ecosistemas de montaña. UNESCO, pp. 37–98.

Bustos-Obregón E., Esveile C., Contreras J., Maurer I., Sarabia L. (2006). Effects of chronic simulated hypobaric hypoxia on mouse spermatogenesis. Int. J. Morphol., 24: 481–488.

Campbell R.C., Dott H.M., Glover T.D. (1956). Nigrosin eosin as a stain for differentiating live and dead spermatozoa. J. Agricult. Sci., 48: 1–8.

Cheviron Z.A., Brumfield R.T. (2012). Genomic insights into adaptation to high-altitude environments. Heredity, 108: 354–361.

Cheviron Z.A., Whitehead A., Brumfield R.T. (2008). Transcriptomic variation and plasticity in rufous-collared sparrows (*Zonotrichia capensis*) along an altitudinal gradient. Mol. Ecol., 17: 4556–4569.

Chung J.Y., Kim Y.J., Kim J.Y., Lee S.G., Park J.E., Kim R.W., Yoon Y.D., Yoo K.S., Yoo Y.H., Kim J.M. (2011). Benzo[*a*]pyrene reduces testosterone production in rat Leydig cells via a direct disturbance of testicular steroidogenic machinery. Environ. Health Perspect., 119: 1569–1574.

Cikutovic M., Fuentes N., Bustos-Oregon E. (2009). Effect of intermittent hypoxia on the reproduction of rats exposed to high altitude in Chilean Altiplano. High Alt. Med. Biol., 10: 357–363.

Çoksevim B., Narin F., Yaba G. (2006). The effects of mid-altitude on endocrine profile. Biol. Sport, 23: 33–40.

Colon E., Svechnikov K.V., Carlsson-Skwirut C., Bang P., Soder O. (2005). Stimulation of steroidogenesis in immature rat Leydig cell evoked by interleukin-1alpha is potentiated by growth hormone and insulin-like growth factors. Endocrinol., 146: 221–230.

Dombrowicz D., Hooghe-Peters E.L., Gothot A., Sente B., Vanhaelst L., Closset J., Hennen G. (1992). Cellular localization of IGF I and IGF II mRNAs in immature hypophysectomized rat testis and epididymis after *in vivo* hormonal treatment. Arch. Int. Physiol. Biochim. Biophys., 100: 303–308.

Fagundes N.J.R., Salzano F.M., Batzer M.A., Deininger P.L., Bonatto S.L. (2005). Worldwide genetic variation at the 3-UTR region of the LDLR gene: possible influence of natural selection. Ann. Hum. Genetics, 69: 389–400.

Farias J.G., Bustos-Obregén E., Orellana R., Bucarey J.L., Quiroz E., Reyes J.G. (2005). Effects of chronic hypobaric hypoxia on testis histology and round spermatid oxidative metabolism. Andrologia, 37: 47–52.

Garcia-Hjarles M.A. (1989). Sperm count and seminal biochemistry of high altitude inhabitants and patients with chronic altitude sickness. Arch. Biol. Med. Exp., 22: 61–67.

Gasco M., Rubio J., Chung A., Villegas L., Gonzales G.F. (2003). Effect of high altitude exposure on spermatogenesis and epididymal sperm count in male rats. Andrologia, 35: 368–374.

Ge R.S., Dong Q., Sottas C.M., Papadopoulos V., Zirkin B.R., Hardy M.P. (2006). In search of rat stem Leydig cell: identification, isolation, and lineage-specific development. Proc. Natl. Acad. Sci. USA, 103: 2719–2724**.**

Gosney J.R. (1984). Effects of hypobaric hypoxia on the Leydig cell population of the testis of the rat. J. Endocrinol., 103: 59–62.

Hanukoglu I. (1992). Steroidogenic enzymes: structure, function, and role in regulation of steroid hormone biosynthesis. J. Steroid. Biochem. Mol. Biol., 43: 779–804.

Leach R.M., Treacher D.F. (1998). Oxygen transport-2. Tissue hypoxia. British Med. J., 317: 1370–1373.

Luo D.Y., Yang G., Liu J.J., Yang Y.R., Dong Q. (2011). Effects of varicocele on testosterone, apoptosis and expression of StAR mRNA in rat Leydig cells. Asian. J. Androl., 13: 287–291.

Myers D.A., Hyatt K., Mlynarczyk M., Brid I.M., Ducsay C. (2005). Long-term hypoxia represses the expression of key genes regulating cortisol biosynthesis in the near-term ovine fetus. Am. J. Physiol. Regul. Integr. Comp. Physiol., 289: R1707-R1714.

Nakayama Y., Yamamoto T., Abe S.I. (1999). IGF-I, IGF-II and insulin promote differentiation of spermatogonia to primary spermatocytes in organ culture of newt testes. Int. J. Dev. Biol., 43: 343–347.

Nelson M.L., Cons J.M., Hodgdon G.E. (1975). Effects of simulated high altitude (3800 m) on reproductive function in the pregnant rat. Environ Physiol Biochem., 5: 65–72.

Okumura A., Fuse H., Kawauchi Y., Mizuno I., Akashi T. (2003). Changes in male reproductive function after high altitude mountaineering. High Alt. Med. Biol., 4: 349–353.

Park E.K., Chu J.P., Parkinson T., Cockrem J.F., Han K.S., Blair H.T., Kim T.Y., Yoon J.T., Lee Y.S. (2010). Rams genetically superior for IGF-I do not exhibit improved male reproductive traits. Anim. Reprod. Sci., 118: 223–230.

Parraguez V.H., Urquieta B., Perez L., Castellaro G., Reyes M.D.L., Torres-Rovira L., Aguado-Martinez A., Astiz S., Gonzalez-Bulnes A. (2013). Fertility in a high-altitude environment is compromised by luteal dysfunction: the relative roles of hypoxia and oxidative stress. Reprod. Biol. Endocrinol., 11: 24–35.

Peters M.A.J., Mol J.A., van Wolferen M.E., Oosterlaken-Dijksterhuis M.A., Teerds K.J., van Sluijs F.J. (2003). Expression of insulin-like growth factor (IGF) system and steroidogenic enzymes in canine testis tumors. Reprod. Biol. Endocrinol., 1, p. 22.

Reeves J.T., Weil J.V. (2001). Chronic mountain sickness. A view from the crow's nest. Adv. Exp. Med. Biol., 502: 419–437.

Saxena D.K. (1995). Effect of hypoxia by intermittent altitude exposure on semen characteristics and testicular morphology of male rhesus monkeys. Int. J. Biometeorol., 38: 137–140.

Vargas V.E., Kaushal K.M., Monau T., Myers D.A., Ducsay C. A. (2011). Long-term hypoxia enhances cortisol biosynthesis in near-term ovine fetal adrenal cortical cells. Reprod. Sci., 18: 277–285.

Velickovic L.J., Stefanovic V. (2014). Hypoxia and spermatogenesis. Int Urol Nephrol., 46: 887– 894.

Verratti V., Giulio D. (2012). High-altitude hypoxia and reproduction: is there an environmental limit to the human male reproductive system? Sport Sci. Health, 7: 39–40.

Verratti V., Berardinelli F., Di Giulio C., Bosco G., Cacchio M., Pellicciotta M., Nicolai M., Martinotti S., Tenaglia R. (2008). Evidence that chronic hypoxia causes reversible impairment on male fertility. Asian J. Androl., 10: 602–606.

Wang G.M., O'Shaughnessy P.J., Chubb C., Robaire B., Hardy M.P. (2003). Effects of insulin-like growth factor I on steroidogenic enzyme expression levels in mouse Leydig cells. Endocrinol., 144: 5058–5064.

Wang H., Wang Q., Zhao X.F., Liu P., Meng X.H., Yu T., Ji Y.L., Zhang H., Zhang C., Zhang Y., Xu D.X. (2010). Cypermethrin exposure during puberty disrupts testosterone synthesis via down regulating StAR in mouse testes. Arch. Toxicol., 84: 53–61.

Zhang G.L., Feng Y., Dai D.Z., Cheng Y.S., Zhang C., Dai Y. (2012). CPU86017-RS attenuate hypoxia-induced testicular dysfunction in mice by normalizing androgen biosynthesis genes and pro-inflammatory cytokines. Acta Pharmacol. Sinic., 33: 470–478.

Zhang G.L., Dai D.Z., Zhang C., Dai Y. (2013). Apocynin and raisanberine alleviate intermittent hypoxia induced abnormal StAR and 3β-HSD and low testosterone by suppressing endoplasmic reticulum stress and activated p66Shc in rat testes. Reprod. Toxicol., 36: 60–70.

Received: 6 III 2014
Accepted: 9 XII 2014