

高原牦牛隐睾组织结构特征

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摘要: 观察成年牦牛隐睾的组织结构特点, 分析高原环境对其生殖微环境的影响。应用 HE、Masson's、Gomori's 特殊染色以及免疫组织化学方法和透射电镜观察比较成年牦牛隐睾与单侧正常睾丸、正常睾丸组织结构特点, 进而用 IPP 图像分析软件进行定量统计。与正常组睾丸相比, 隐睾生精小管管径极显著减小 ($P < 0.01$), 基膜增厚, 腔内生殖细胞丢失, 散在少数幼稚型 Sertoli 细胞, 胞内线粒体变性且含有大小不等的脂褐素颗粒; 间质内胶原纤维增生, 间质/管腔面积比极显著增大 ($P < 0.01$), Leydig 细胞数量减少, 胞内线粒体肿胀, 间质血管数量减少, 管壁增厚皱缩, 血管内皮细胞内含大量脂褐素颗粒; 睾丸实质部分钙化。单侧正常组睾丸生精上皮细胞为 3~4 层, Sertoli 细胞发育成熟, 少见初级精母细胞及精子; 间质/管腔面积比与正常睾丸无明显差异 ($P > 0.05$), Leydig 细胞数量较多, 内质网丰富呈一定扩张状态, 线粒体减少。免疫组织化学显示, VEGF 及 VEGFR2 在隐睾组与单侧正常组、正常组睾丸均表达于 Sertoli 细胞、Leydig 细胞及各级生精细胞, 血管内皮细胞偶见表达; 隐睾组 VEGF 表达量较正常组、单侧正常组睾丸明显下降 ($P < 0.05$), VEGFR2 表达量明显高于正常组与单侧正常组 ($P < 0.05$); 单侧正常组 VEGF 及 VEGFR2 表达量较正常组均无明显差异 ($P > 0.05$)。高原低氧环境, 牦牛隐睾血管发育受阻, 组织出现不同程度的纤维化和局部钙化, Sertoli 细胞发育异常严重影响生精功能; 单侧正常睾丸 Leydig 细胞数量增加, 而其中线粒体数量减少, 发育程度较正常组织有所降低, 隐睾组织中 VEGF 与 VEGFR2 可能参与调节双侧睾丸生精抑制作用。

关键词: 牦牛; 隐睾; 组织结构; 血管内皮生长因子; 血管内皮生长因子受体 2

中图分类号: S852.16

文献标志码: A

文章编号: 0366-6964(2015)12-2282-09

The Histologic Characteristics of Yak Cryptorchidism

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Abstract: The aim of this study was to observe the histologic characteristics of adult yaks cryptorchidism and analyze the influence of plateau environment on the cryptorchidism reproductive microenvironment. Masson's and Gomori's staining, histochemistry and transmission electron methods was used to characterize the microstructure and ultrastructure of cryptorchidism, normal testis and unilateral normal testis in yak, and IPP (Image-Pro Plus) statistics method was used to quantitative statistics. The volume and weight of yak cryptorchidism were lesser than the normal testis and companied with depression of the lumen, the basement membrane was thickened, as well as the diameter of seminiferous tubule was significantly reduced ($P < 0.01$). The spermatogenic cells desquamated and immature Sertoli cells were scattered in the tubule. Obviously, there were mitochondria degeneration and many lipofuscin granules with different sizes in the Sertoli cells. Besides, the area ratio of interstitial to lumen was significantly increased ($P < 0.01$), the numbers of Leydig cells were decreased, and the mitochondria in it was swelling. There were partly calcifi-

收稿日期: 2015-04-02

基金项目: 国家自然科学基金项目(31160488); 甘肃省自然科学基金(145RJZA223)

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cation was observed in parenchyma and not only the quantity of interstitial vascular was reduced but the vessel wall was thicken and shrunken. The seminiferous epithelium of unilateral normal testis were 3-4 layers with matured Sertoli cells, the primary spermatocytes and spermatozoa were rarely seen, and there was no difference with the normal testis in interstitial/lumen area ratios ($P > 0.05$). The number of Leydig cells increased and the endoplasmic reticulum in it were represented as an loose and vesiculated network and the number of mitochondria were decreased. VEGF and its receptor(VEGFR2) immunoreactivities were abundantly distributed in the gonads of cryptorchidism, normal testis and unilateral normal testis, mainly associated with Sertoli cells, Leydig cells and spermatogenic cells, and weakly present throughout the vascular endothelial cells. Immunostaining analysis appeared that the relative expression of VEGF in cryptorchidism was significantly decreased than in normal testis and unilateral normal testis ($P < 0.05$), by contrast, the expression of VEGFR2 in cryptorchidism was stronger than the other two groups. But the expression of VEGF and VEGFR2 had no significant difference between the normal and unilateral normal testis ($P > 0.05$). Taken together, in plateau environment, the cryptorchidism vascular of Yak was suffocated, the seminiferous function was seriously affected by the different degree of fibrosis and calcification in parenchyma and the dysplasia of the Sertoli cell; but in unilateral normal testis, accompanying the number of Leydig cells increased, the number of mitochondria decreased and also growth degree was decreased compared with the normal tissue. Our results suggest that the VEGF and VEGFR2 may serve as regulators to participate in the bilateral testicular spermatogenic suppression effect.

Key words: yak; cryptorchidism; immunohistochemistry; VEGF; VEGFR2

隐睾是一种常见的生殖系统疾病,是雄性不育的主要原因之一。隐睾引起睾丸缺血缺氧,致使生精环境改变及生精细胞凋亡、睾丸纤维化,严重时可引起睾丸钙化等退行性改变^[1-2]。隐睾还可导致睾丸癌发病率升高,最终影响生育能力^[3]。目前关于隐睾引起不育的研究资料主要集中于人类、啮齿类实验动物(如大鼠、小鼠)等,对于高海拔地区人或动物的研究资料很少。据报道,隐睾可致睾丸毛细血管生成或退行性改变,血管内皮层增厚,阻碍与间质的物质交换^[4]。高原环境中藏绵羊等动物睾丸小叶的微血管分布有显著的高海拔低氧适应特征^[5]。X. Y. Wu 等^[6]对牦牛血管内皮生长因子基因研究表明,血管内皮生长因子作为一种血管生成的关键调节器和一个内皮细胞的有丝分裂原,在高海拔适应性中起着重要作用。研究报道^[7], VEGF 及其受体(vascular endothelial growth factor receptor, VEGFR)在人体许多与生殖有关的细胞上均有表达,是体内与生殖有关的重要生长因子之一。高海拔低氧小鼠应激模型研究发现,缺氧条件下血浆 VEGF 浓度增加^[8]; VEGF 及 VEGFR 通过旁分泌或是自分泌的形式与激素等其他因子共同作用于辜

丸间质毛细血管,来增加其新生血管形成和通透性,提高机体耐受缺氧的能力^[9]。牦牛长期生活在高海拔地区,是典型的季节性发情动物,其繁殖能力较低。近年来,关于成年牦牛睾丸形态及生物学方面的研究较多,但是对牦牛隐睾的组织学特征、血管分布变化、VEGF 及 VEGFR 表达研究尚未见报道。因此,研究高原低氧环境中牦牛隐睾的组织学特点,进一步分析 VEGF 及其受体表达变化,有助于深入了解其生殖生理特点,为高原地区动物隐睾与生殖生理的研究提供形态学参考。

1 材料与方法

1.1 试验动物

样品采自青海省大通牧区,冬季,健康性成熟 3~4 岁牦牛,隐睾位于腹股沟管或腹腔内,外科手术取出睾丸。共 20 例,正常睾丸 14 例,隐睾 6 例,其中单侧隐睾 4 例。以 4% 中性福尔马林溶液固定 3 d 后备用。组织样分正常睾丸组、隐睾组、单侧正常睾丸组进行研究。

1.2 主要试剂

VEGF 兔抗鼠多克隆抗体(bs-1313R)和 VEG-

FR2 兔抗鼠多克隆抗体(bs-0565R)购自北京博奥森生物技术有限公司;免疫组化染色试剂盒(sp-0023,由美国 ZYMED 公司生产)购自北京博奥森生物技术有限公司;DAB 显色试剂盒(ZLI-9018)和 APES 防脱玻片(ZLI-9502)购自北京中杉金桥生物技术有限公司。

1.3 普通切片制备

新鲜组织样品称重测量,记录解剖数据,甲醛溶液(福尔马林)固定,常规石蜡包埋,连续切片(片厚 $5\ \mu\text{m}$),相邻切片分为 6 套,其中 2 套分别用于 VEGF 和 VEGFR2 免疫组织化学 SP 法染色,剩下 4 套用于组织化学 HE、Masson's、Gomori's 染色及阴性对照。

1.4 组织化学染色法

切片脱水、脱蜡后分别进行 HE、Masson's、Gomori's 组织化学染色,之后继续进行脱水、透明,然后封片、观察、拍照。HE 染色,细胞核为蓝紫色,其余均为红色;Masson's 三色染色(亮绿),胶原纤维呈现蓝绿色,细胞核呈现灰黑或灰蓝色,红细胞呈红色;Gomori's 银染显示网状纤维为灰色,苏木素-伊红复染后呈棕红色。

1.5 透射电镜观察

2.5%戊二醛、2%多聚甲醛磷酸缓冲液与 1%

锇酸磷酸缓冲液双重固定组织,梯度酒精脱水,氧化丙烯置换,Epon812 树脂包埋,超薄切片,醋酸-柠檬酸铅双重染色,进行观察。

1.6 免疫组织化学 SP 法检测 VEGF 和 VEGFR2 分布及图像分析

切片在 NIKON ECLIPSE 80i 显微摄像系统进行照相。参照袁莉刚等^[10]在不同年龄牦牛睾丸研究中的方法,每组随机选取 5 张切片,每张切片随机选取 6 个不重复视野($\times 400$),用 Image Pro Plus 6.0 软件统计每个视野下生精小管直径、管腔面积、间质面积。统计免疫组织化学染色结果中每个视野下阳性信号表达平均吸光度,用 SPSS 21.0 统计软件对数据进行配对 t 检验,结果用 $\bar{x} \pm s_{\bar{x}}$ 表示, $P < 0.05$ 为差异显著, $P < 0.01$ 为差异极显著。

2 结果

2.1 牦牛隐睾、单侧正常睾丸与正常睾丸解剖特征比较

隐睾位于腹股沟管或腹腔内,睾丸大小及重量均较正常睾丸极显著降低($P < 0.01$),质地较硬,眼观发黄,切面有黄白色黏液流出,严重者钙化明显;单侧隐睾,正常下降侧睾丸重量及大小指数较正常组均略小($P > 0.05$),但质地柔软、饱满(表 1)。

表 1 牦牛隐睾、单侧正常睾丸与正常睾丸解剖特征指数比较

Table 1 The comparison of the anatomical characteristic index of cryptorchidism, unilateral normal testicles, normal testicles in yak

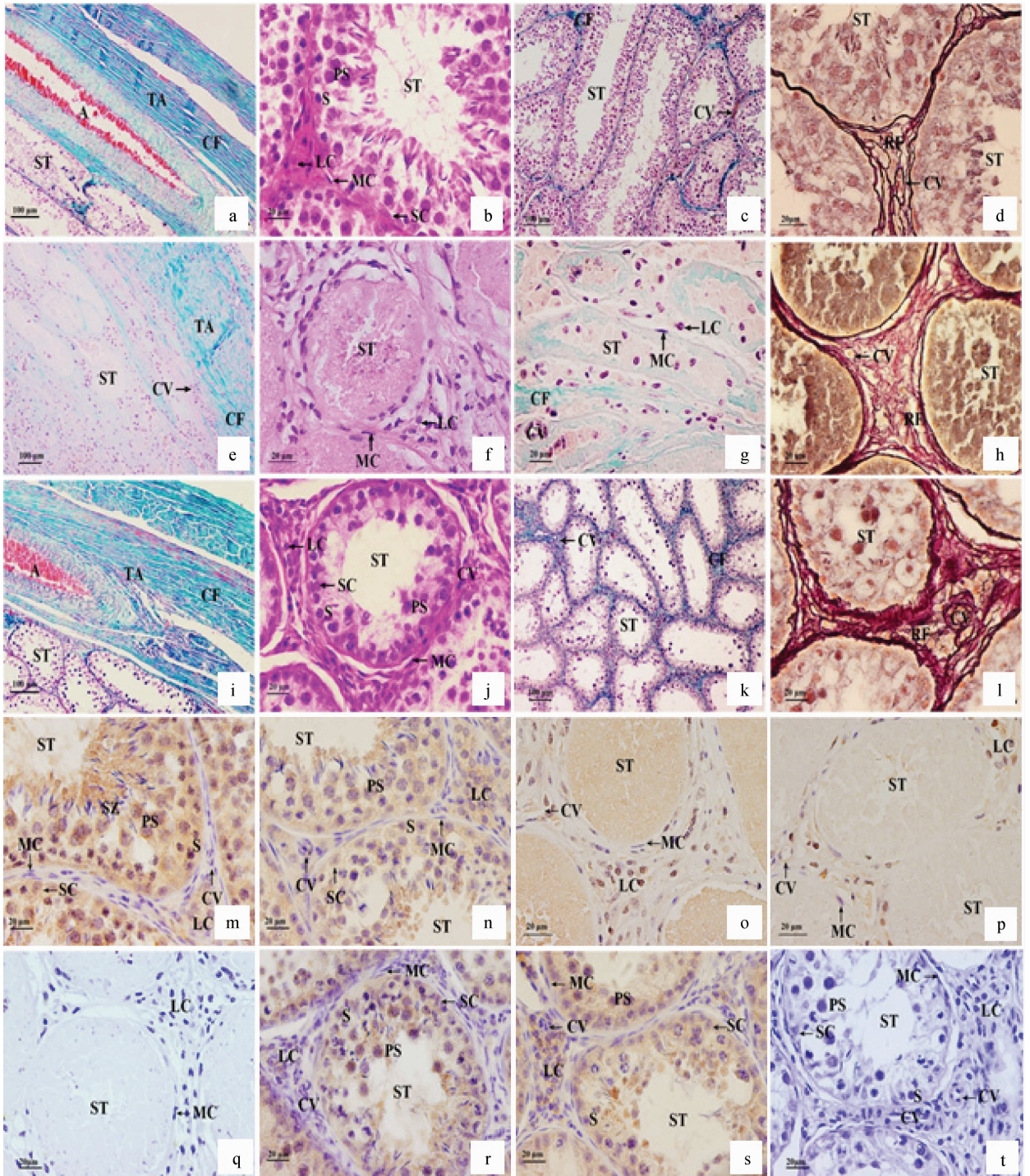
组别 Group	长径/cm Olichio-diameter	横径/cm Brachy-diameter	厚径/cm Hadro-diameter	重量/g Weight
正常组 The normal group	5.967 \pm 0.168 ^a	4.067 \pm 0.069 ^a	3.267 \pm 0.077 ^a	95.425 \pm 1.814 ^a
隐睾组 The cryptorchidism group	2.911 \pm 0.208 ^{b*}	2.307 \pm 0.115 ^b	1.867 \pm 0.038 ^b	14.301 \pm 1.278 ^{b*}
单侧正常组 The unilateral normal group	5.553 \pm 0.416 ^a	3.724 \pm 0.240 ^a	3.033 \pm 0.117 ^a	89.425 \pm 2.305 ^a

同列标有不同字母的差异显著($P < 0.05$),标有相同字母的差异不显著($P > 0.05$),标有*的差异极显著($P < 0.01$)。下表同 Different letters in the column means difference between the groups($P < 0.05$),same letters mean no difference between groups ($P > 0.05$),* means significant difference between groups($P < 0.01$). The same as below

2.2 牦牛隐睾、单侧正常睾丸及正常睾丸的组织结构特点

光镜下观察,正常睾丸被膜结构致密,富含胶原纤维、血管(图 1a);基膜平整,生精小管发育良好,小管平均直径(263.880 ± 3.560) μm ,6~7 层生精细胞,精子数量较多(图 1b);间质内胶原及网状纤维、血管均丰富(图 1c、图 1d)。隐睾内胶原纤维增生,被膜内少见小血管,血管减少,管壁皱缩,管腔缩

小,仅可见微血管(图 1e);生精小管平均直径较正常组极显著减小($P < 0.01$,表 2),基膜增厚内陷,仅见少数 Sertoli 细胞散在于小管内,生精细胞缺失(图 1f);Leydig 细胞减少,间质/管腔面积比较正常组极显著增大($P < 0.01$,表 2),网状纤维无明显变化(图 1g、图 1h);靠近被膜处尚有间质和生精小管结构存在,实质深部近纵隔处明显钙化,组织结构不清晰。单侧正常睾丸被膜发育完整(图 1i),生精小



a. 牦牛正常组睾丸被膜, Masson's 染色, 标尺示 $100\ \mu\text{m}$; b. 牦牛正常组睾丸实质, HE 染色, 标尺示 $20\ \mu\text{m}$; c. 牦牛正常组睾丸实质, Masson's 染色, 标尺示 $100\ \mu\text{m}$; d. 牦牛正常组睾丸实质, Gomori's 染色, 标尺示 $20\ \mu\text{m}$; e. 牦牛隐睾组睾丸被膜, Masson's 染色, 标尺示 $100\ \mu\text{m}$; f. 牦牛隐睾组睾丸实质, HE 染色, 标尺示 $20\ \mu\text{m}$; g. 牦牛隐睾组睾丸实质, Masson's 染色, 标尺示 $100\ \mu\text{m}$; h. 牦牛隐睾组睾丸实质, Gomori's 染色, 标尺示 $20\ \mu\text{m}$; i. 牦牛单侧正常组睾丸被膜, Masson's 染色, 标尺示 $100\ \mu\text{m}$; j. 牦牛单侧正常组睾丸实质, HE 染色, 标尺示 $20\ \mu\text{m}$; k. 牦牛单侧正常组睾丸实质, Masson's 染色, 标尺示 $100\ \mu\text{m}$; l. 牦牛单侧正常组睾丸实质, Gomori's 染色, 标尺示 $20\ \mu\text{m}$; m. VEGF 在牦牛正常组睾丸中的表达, 免疫组化染色, 标尺示 $20\ \mu\text{m}$; n. VEGFR2 在牦牛正常组睾丸中的表达, 免疫组化染色, 标尺示 $20\ \mu\text{m}$; o. VEGF 在牦牛隐睾组睾丸中的表达, 免疫组化染色, 标尺示 $20\ \mu\text{m}$; p. VEGFR2 在牦牛隐睾组睾丸中的表达, 免疫组化染色, 标尺示 $20\ \mu\text{m}$; q. 牦牛隐睾组睾丸, 阴性对照, 标尺示 $20\ \mu\text{m}$; r. VEGF 在牦牛单侧正常组睾丸中的表达, 免疫组化染色, 标尺示 $20\ \mu\text{m}$; s. VEGFR2 在牦牛单侧正常组睾丸中的表达, 免疫组化染色, 标尺示 $20\ \mu\text{m}$; t. 牦牛单侧正常组睾丸, 阴性对照, 标尺示 $20\ \mu\text{m}$; ST. 生精小管; TA. 被膜; A. 动脉; CV. 微血管; MC. 肌样细胞; LC. 间质细胞; SC. Sertoli 细胞; S. 精原细胞; PS. 初级精母细胞

a. The structure of capsule in normal yak testis, Masson's staining, bar=100 μm ; b. The structure of parenchyma in normal yak testis, HE staining, bar=20 μm ; c. The structure of parenchyma in normal yak testis, Masson's staining, bar=100 μm ; d. The structure of parenchyma in normal yak testis, Gomori's staining, bar=20 μm ; e. The structure of capsule in cryptorchidism yak testis, Masson's staining, bar=100 μm ; f. The structure of parenchyma in cryptorchidism yak testis, HE staining, bar=20 μm ; g. The structure of parenchyma in cryptorchidism yak testis, Masson's staining, bar=20 μm ; h. The structure of parenchyma in cryptorchidism yak testis, Gomori's staining, bar=20 μm ; i. The structure of capsule in unilateral normal yak testis, Masson's staining, bar=100 μm ; j. The structure of parenchyma in unilateral normal yak testis, HE staining, bar=20 μm ; k. The structure of parenchyma in unilateral normal yak testis, Masson's staining, bar=100 μm ; l. The structure of parenchyma in unilateral normal yak testis, Gomori's staining, bar=20 μm ; m. The expression of VEGF in normal yak testis, immunohistochemical staining, bar=20 μm ; n. The expression of VEGFR2 in normal yak testis, immunohistochemical staining, bar=20 μm ; o. The expression of VEGF in cryptorchidism yak testis, immunohistochemical staining, bar=20 μm ; p. The expression of VEGFR2 in cryptorchidism yak testis, immunohistochemical staining, bar=20 μm ; q. The control of VEGF/VEGFR2 in cryptorchidism yak testis, bar=20 μm ; r. The expression of VEGF in unilateral normal yak testis, immunohistochemical staining, bar=20 μm ; s. The expression of VEGFR2 in unilateral normal yak testis, immunohistochemical staining, bar=20 μm ; t. The control of VEGF/VEGFR2 in unilateral normal yak testis, bar=20 μm ; ST. Seminiferous tubule; TA. Testicular albuginea; A. Artery; CV. Capillary vascular; CF. Collagen fiber; RF. Reticular fiber; MC. Muscle-like cell; LC. Leydig cell; SC. Sertoli cell; S. Spermatogonia; PS. Primary spermatocyte

图 1 牦牛隐睾、单侧正常睾丸与正常睾丸的组织结构比较

Fig. 1 The comparison results of the organizational structure of cryptorchidism, unilateral normal testicles, normal testicles in yak

表 2 牦牛隐睾、单侧正常睾丸与正常睾丸生精小管特征指数比较

Table 2 The comparison of the seminiferous tubule characteristic index of cryptorchidism, unilateral normal testicles, normal testicles in yak

组别 Group	管腔平均直径/ μm The average diameter of the seminiferous tubules	管腔面积/ μm^2 Bureaucratic area	间质面积/ μm^2 Interstitial area	间质面积/管腔面积 The ratio of interstitial area and bureaucratic area
正常组 The normal group	263.880 \pm 3.560 ^a	29 796.091 \pm 912.670 ^a	6 202.871 \pm 1 075.104 ^c	0.214 \pm 0.039 ^b
隐睾组 The cryptorchidism group	149.519 \pm 2.682 ^{c*}	15 514.791 \pm 969.705 ^{c*}	20 484.170 \pm 897.278 ^{a*}	1.557 \pm 0.376 ^{a*}
单侧正常组 The unilateral normal group	205.225 \pm 4.799 ^b	27 862.731 \pm 455.800 ^b	8 136.228 \pm 428.837 ^b	0.294 \pm 0.021 ^b

管发育不良,小管平均直径较正常组显著减小($P < 0.05$,表 2),生精上皮细胞 3~4 层,生精细胞数目减少,精原细胞层排列有序,初级精母细胞散在,部分生精细胞脱落到管腔,偶见精子(图 1j);间质紧密,胶原纤维与血管丰富,间质/管腔面积比与正常睾丸无明显差异($P > 0.05$,表 2,图 1k、图 1l),但略高于正常组。

2.3 VEGF 及其受体免疫组织化学分布特征

正常组睾丸中,VEGF 表达于各级生精细胞(图 1m)以及 Sertoli 细胞和 Leydig 细胞,强表达于长形精子,小血管内皮偶见阳性表达,微血管及肌样细胞未见表达;其受体 VEGFR2,同样表达于各级生精细胞(图 1n),长形精子及 Sertoli 细胞, Leydig 细胞强表达。隐睾组中,VEGF 及其受体 VEGFR2 均表

达于残存的各级生精细胞及少数 Sertoli 细胞、Leydig 细胞中(图 1o、图 1p);且 VEGFR2 在 Leydig 细胞同样强表达,在萎缩的生精小管中广泛强表达。单侧正常组睾丸表达与正常组相同(图 1r、图 1s)。

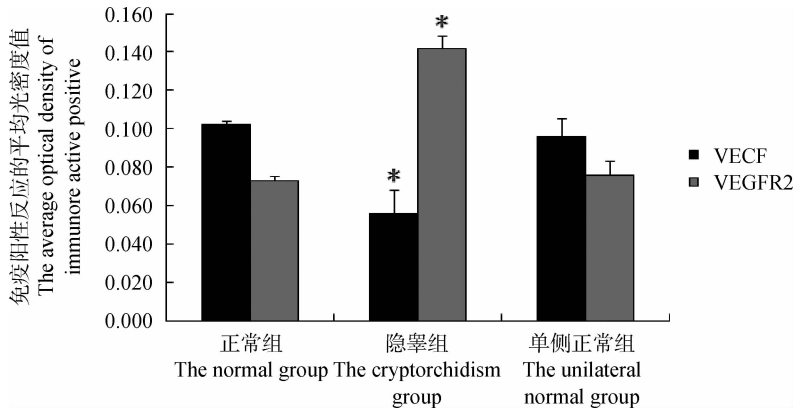
2.4 VEGF 及其受体免疫组织化学检测结果对比分析

免疫组织化学图像分析结果(表 3,图 2)显示,隐睾组 VEGF 表达定位与正常组相同,表达量显著低于正常组($P < 0.05$);VEGFR2 表达显著高于正常组及单侧正常组($P < 0.05$)。单侧正常睾丸组,VEGF 及 VEGFR2 表达情况与正常组无明显差异,VEGF 表达显著高于隐睾组($P < 0.05$),VEGFR2 表达显著低于隐睾组($P < 0.05$)。

表 3 牦牛隐睾、单侧正常睾丸及正常睾丸 VEGF 及其受体 VEGFR2 平均光密度统计

Table 3 The statistical result of the average optical density of VEGF and VEGFR2 in cryptorchidism, unilateral normal testicles, normal testicles of yak

组别 Group	VEGF 平均光密度 The average optical density of VEGF	VEGFR2 平均光密度 The average optical density of VEGFR2
正常组 The normal group	0.102 ± 0.002 ^a	0.073 ± 0.002 ^b
隐睾组 The cryptorchidism group	0.056 ± 0.012 ^b	0.142 ± 0.006 ^a
单侧正常组 The unilateral normal group	0.096 ± 0.009 ^a	0.076 ± 0.007 ^b



标有“*”的组间差异显著($P < 0.05$)

“*” mean difference between the groups($P < 0.05$)

图 2 VEGF 及 VEGFR2 在牦牛隐睾、单侧正常睾丸及正常睾丸中免疫组织化学表达

Fig 2 The Immunohistochemistry expression of VEGF and VEGFR2 in cryptorchidism, unilateral normal testicles, normal testicles of yak

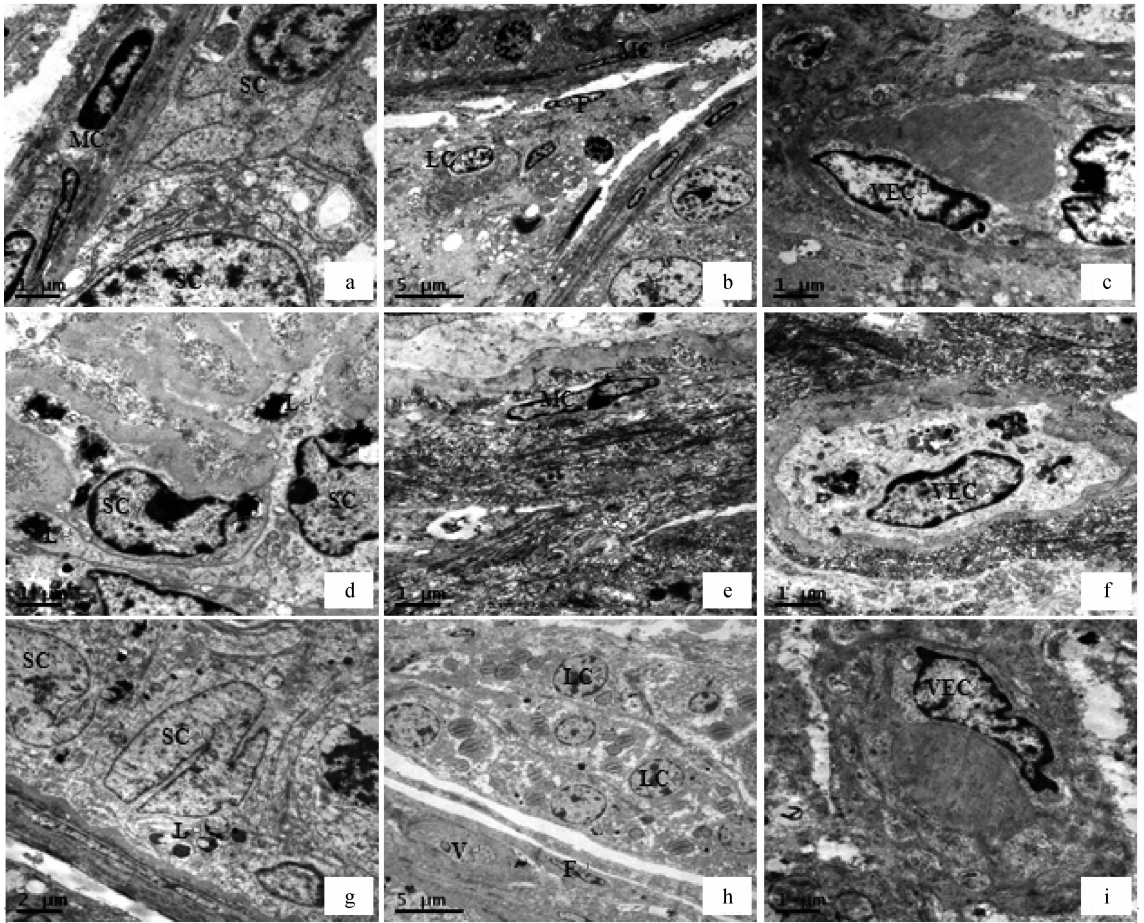
2.5 牦牛隐睾、单侧正常睾丸及正常睾丸组织的超微结构特点

正常组睾丸生精小管基膜平整,胶原纤维分布均匀,细胞发育良好,细胞间连接紧密(图 3a),Sertoli 细胞胞浆内有丰富的内质网与线粒体(图 3b),Leydig 细胞发育成熟,染色质颗粒匀细,核仁致密明显;血管内皮细胞发育良好,管壁平整(图 3c)。隐睾生精小管基膜增厚,上皮细胞不完整,管腔内陷,无生殖细胞,少数幼稚型 Sertoli 细胞核为多边形,有一个或多个核仁,异染色质集合于核膜不同部分,细胞内变性线粒体和脂褐素含量较多或内有大小不等的空泡(图 3d);间质内胶原纤维大量增生,Leydig 细胞数量减少且胞质较少,细胞内线粒体肿胀及大小不等的空泡(图 3e);血管管壁不平整,内皮细胞肿大,内含大量脂褐素,血管周围环绕大量胶原纤维(图 3f)。单侧正常组睾丸与正常组大致相似,但其 Leydig 细胞数量较多,细胞质中内质网丰

富呈一定扩张状态,线粒体数量较少,但是个体严重肿胀(图 3g-i)。

3 讨论

隐睾是雄性生殖系统常见的先天性畸形,双侧或单侧隐睾时睾丸处于高温、缺血、缺氧等非正常状态,进而影响生精功能^[9]。A. García Guerra 等^[11]研究表明睾丸重量的增加与生精上皮发育有直接关系,其大小可直接或间接反映患畜的生育能力。本研究中年龄段牦牛隐睾体积及重量均显著低于正常组,生精细胞严重缺失;单侧正常组睾丸体积也有所减小,生精细胞发育迟缓或处于停滞状态,提示隐睾及单侧正常睾丸发育程度均有所降低。A. Suskind 等^[12]发现睾丸纤维化程度与其功能呈负相关。本研究隐睾生精小管基膜增厚内陷且管径明显减小;间质组织胶原纤维异常增生,间质/管腔面积比较正常组显著增加,表明隐睾局部有纤维化趋



a. 牦牛正常组睾丸实质超微结构, 标尺示 $1 \mu\text{m}$; b. 牦牛正常组睾丸间质超微结构, 标尺示 $5 \mu\text{m}$; c. 牦牛正常组睾丸间质血管超微结构, 标尺示 $1 \mu\text{m}$; d. 牦牛隐睾组睾丸实质超微结构, 标尺示 $1 \mu\text{m}$; e. 牦牛隐睾组睾丸间质超微结构, 标尺示 $1 \mu\text{m}$; f. 牦牛隐睾组睾丸间质血管超微结构, 标尺示 $1 \mu\text{m}$; g. 牦牛单侧正常组睾丸实质超微结构, 标尺示 $2 \mu\text{m}$; h. 牦牛单侧正常组睾丸间质超微结构, 标尺示 $5 \mu\text{m}$; i. 牦牛单侧正常组睾丸间质血管超微结构, 标尺示 $1 \mu\text{m}$; F. 成纤维细胞; L. 脂褐素; LC. 间质细胞; MC. 肌样细胞; SC. Sertoli 细胞; V. 血管; VEC. 血管内皮细胞

a. The ultrastructure of parenchyma in normal yak testis; bar = $1 \mu\text{m}$; b. The ultrastructure of leydig in normal yak testis; bar = $5 \mu\text{m}$; c. The ultrastructure of vascular in normal yak testis; bar = $1 \mu\text{m}$; d. The ultrastructure of parenchyma in cryptorchidism yak testis, bar = $1 \mu\text{m}$; e. The ultrastructure of leydig in cryptorchidism yak testis, bar = $1 \mu\text{m}$; f. The ultrastructure of vascular in cryptorchidism yak testis, bar = $1 \mu\text{m}$; g. The ultrastructure of parenchyma in unilateral normal yak testis, bar = $2 \mu\text{m}$; h. The ultrastructure of leydig in unilateral normal yak testis, bar = $5 \mu\text{m}$; i. The ultrastructure of vascular in unilateral normal yak testis, bar = $1 \mu\text{m}$; F. Fibroblast; L. Lipofuscin; LC. Leydig cell; MC. Muscle-like cell; SC. Sertoli cell; V. Vascular; VEC. Vascular endothelial cell

图 3 牦牛隐睾、正常睾丸及单侧正常睾丸组织的超微结构比较

Fig. 3 The comparison results of the ultrastructure of cryptorchidism, unilateral normal testicles, normal testicles in yak

势。研究报道^[13], 缺氧时有机体局部氧化与抗氧化作用失衡, 引起氧化应激, 导致组织损伤。M. Ott 等^[14]研究发现, 氧化应激介导线粒体变性, 使其功能受损, 最终导致细胞凋亡。研究表明^[15], 隐睾内细胞增殖/凋亡率降低, 生殖细胞缺失。本研究中隐睾仅存有少数幼稚型 Sertoli 细胞, 且细胞质内存在大量变性线粒体和脂褐素, 表明 Sertoli 细胞发育受阻, 可能是诱导生殖细胞减少的主要原因之一。

研究表明^[13], 缺氧引起氧化应激, 导致组织损

伤。此外, 氧化应激介导并参与血管钙化, 血管内皮细胞受损^[16]。研究显示^[4], 猪隐睾可致睾丸毛细血管生成或退行性改变, 血管内皮层增厚进而阻碍与间质之间的物质交换。M. Chihara 等^[17]发现热应激能引起小鼠睾丸的钙化现象, 但是耐热性小鼠睾丸比实验性小鼠隐睾钙化程度轻, 认为睾丸组织钙化主要与不同品种小鼠机体内钙化抑制蛋白 mRNA 水平不同有关。本研究中隐睾实质深部有钙化现象, 生精小管基膜增厚, 血管内皮细胞肿大,

细胞内脂褐素较多,表明细胞物质交换受阻,且血管明显减少,造成组织局部严重缺氧、缺血,最终引起营养不良性钙化,在其他高原动物隐睾是否也存在钙化现象,或者与高原动物钙化相关基因表达差异有关,有待于进一步研究。

睾丸局部微环境受到各类细胞因子的调节, VEGF 作为内皮细胞特异性强效有丝分裂原,被公认为是作用最强的血管通透因子,在体内 VEGF 只有通过与其受体相结合才能发挥生物学效应, VEGFR2 是其最主要的受体之一, VEGF 以旁分泌形式作用于间质血管的 VEGFR2,调节毛细血管的通透性,从而参与维持微环境的稳定^[7]。本研究中牦牛正常组睾丸各级生精细胞、Sertoli 细胞和 Leydig 细胞均有 VEGF 及 VEGFR2 表达,这与 VEGF、VEGFR2 在大鼠睾丸中的表达结果基本一致^[7],提示 VEGF 可通过与 VEGFR2 相结合,调节睾丸内分泌活动,为生精细胞的增殖和分化创造条件。研究发现^[18],VEGF 及其受体可通过刺激血管内皮细胞增殖,血管生成及其内皮细胞迁移,增加血管通透性等,在雄性生殖系统中发挥重要作用。本研究结果隐睾内 VEGFR 表达的升高,可能是一种代偿性增加,以便充分有效地利用 VEGF,其机制有待于进一步研究。

大鼠隐睾试验显示^[9],单侧隐睾与其对侧正常睾丸存在相似损害,认为是隐睾异位的持续刺激通过生殖股神经传入到对侧交感神经中枢,反射性地影响对侧睾丸,进而导致其发生退行性变化,引起双侧睾丸生精抑制。本研究中单侧正常睾丸发育迟缓,可能受到对侧隐睾的影响。研究报道^[19],在一些高海拔低氧环境土著生物中,细胞线粒体数目反而减少,长期低氧环境选择下机体会通过提高能量利用效率等措施减少机体能量消耗。S. Dutta 等^[20]研究发现小鼠单侧正常睾丸存在雄激素代偿性生理作用;研究表明^[21],双峰驼隐睾中肽能神经对间质细胞的分泌调控并未明显改变,有助于维持正常的雄激素分泌。本研究中,单侧正常睾丸 Leydig 细胞中线粒体数量减少,可能是有助于降低 Leydig 细胞本身的能量消耗,而 Leydig 细胞数量增加,胞质中内质网丰富呈一定扩张状态,可能有增加睾酮分泌进而维持睾丸功能的趋势。超微结构显示单侧正常组睾丸血管内皮细胞正常发育,提示血管结构未受明显影响。M. Tek 等^[22]发现 VEGF 在改善睾丸损伤,减少细胞凋亡中发挥显著作用。本研究单侧正

常睾丸生殖细胞发育停滞,VEGF 及 VEGFR2 表达与正常组均无明显差异,而 VEGF 明显高于隐睾组,这与朱保平等^[9]发现在大鼠单侧正常睾丸中 VEGF 表达量增加一致,表明 VEGF 及其受体可能通过拮抗双侧睾丸生精抑制作用,对维持一定的生殖机能具有重要意义。

4 结 论

高原低氧环境,牦牛隐睾血管发育受阻,组织出现不同程度的纤维化和局部钙化,Sertoli 细胞发育异常严重影响生精功能;单侧正常睾丸 Leydig 细胞数量增加,而其中线粒体数量减少,发育程度较正常组织有所降低,隐睾组织中 VEGF 与 VEGFR2 可能通过拮抗双侧睾丸生精抑制作用,对维持一定的生殖机能具有重要意义。

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