

奶牛静脉血、胎盘组织、脐静脉血及犊牛静脉血脂联素与犊牛初生重相关性研究

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摘要 为探究奶牛静脉血、胎盘组织、脐静脉血和犊牛静脉血中脂联素之间及与犊牛初生重的相关性,选取规模化养殖场正常分娩奶牛54头,按犊牛初生重将奶牛分为3组:A组, ≤ 40 kg,9头;B组,40~45 kg,25头;C组, ≥ 45 kg,20头。分别采集分娩奶牛颈静脉血、胎盘组织、脐静脉血及犊牛颈静脉血,ELISA法检测血液及胎盘组织脂联素水平,相关分析法分析各样品之间及与犊牛初生重的相关性。奶牛静脉血脂联素水平(29.15 ± 4.02 mg/L)高于脐静脉血(13.79 ± 1.14 mg/L)与犊牛静脉血(13.46 ± 0.94 mg/L)脂联素水平,差异极显著($P < 0.01$),脐静脉血脂联素水平高于犊牛静脉血脂联素水平,但差异性不显著($P > 0.05$);奶牛静脉血脂联素水平与胎盘组织、脐静脉血、犊牛静脉血脂联素水平以及胎盘组织脂联素水平与脐静脉血、犊牛静脉血脂联素水平相关性均不显著($P > 0.05$),脐静脉血脂联素水平与犊牛静脉血脂联素水平极显著正相关($P < 0.01$);犊牛初生重与奶牛静脉血脂联素水平无显著相关性($P > 0.05$),与脐静脉血、胎盘组织脂联素水平极显著正相关($P < 0.01$),与犊牛静脉血脂联素水平显著正相关($P < 0.05$);奶牛静脉血、胎盘组织、脐静脉血脂联素水平及犊牛静脉血脂联素水平在公、母犊间差异均不显著($P > 0.05$)。脐静脉血、胎盘组织脂联素水平对犊牛初生重有非常显著的影响,犊牛静脉血脂联素水平对犊牛初生重具有一定影响,而奶牛静脉血脂联素水平对犊牛初生重的影响不显著;奶牛静脉血、脐静脉血、胎盘组织及犊牛静脉血脂联素水平在公、母犊间差异均不显著。

关键词 脂联素;静脉血;胎盘组织;奶牛;犊牛初生重

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Correlation between adiponectin in cows' venous blood,placenta, cord blood,calves venous blood and calf birth weight

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Abstract The aim of this study was to explore the correlation between the calf birth weight and the adiponectin in cows' venous blood,placenta,cord blood and calves' venous blood.Based on the calf birth weight,54 healthy Chinese

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Holstein cows with normal delivery term were divided into 3 groups: Group A (≤ 40 kg, $n = 9$), Group B (40~45 kg, $n = 25$) and Group C (≥ 45 kg, $n = 20$). ELISA was used to evaluate the level of adiponectin in cows' venous blood, placenta, cord blood and calves' venous blood. The correlation between adiponectin and calf birth weight were analyzed by correlation analysis method for further study. Adiponectin levels in cows' venous blood (29.15 ± 4.02 mg/L) were significantly higher than that in cord blood (13.79 ± 1.14 mg/L) and calves' venous blood (13.46 ± 0.94 mg/L) ($P < 0.01$); Adiponectin levels in cows' cord blood were higher than that in venous blood of calves, but the differences were not significant ($P > 0.05$). Adiponectin levels in cows' venous blood had no significant correlation with that in placenta, cord blood and calves' venous blood ($P > 0.05$) and adiponectin levels in placenta also had no significant correlation with that in cord blood and calves' venous blood ($P > 0.05$). However, adiponectin levels in cord blood had highly significantly positive correlation with that in venous blood of cows ($P < 0.01$). The calf birth weight had no significant correlation with the adiponectin levels in cows' venous blood ($P > 0.05$), while it had highly significantly positive correlation with adiponectin levels in cord blood and placenta ($P < 0.01$) and had significantly positive correlation with adiponectin levels in calves' venous blood ($P < 0.05$). Adiponectin levels in cows' venous blood, placenta, cord blood and calves' venous blood had no significantly differences between male and female calves ($P > 0.05$). In conclusion, adiponectin levels in cows' cord blood and placenta showed highly significant influence on the calf birth weight, adiponectin levels in calves' venous blood influenced calf birth weight to a certain extent, while adiponectin levels in cows' venous blood had no significant influence on the calf birth weight.

Keywords adiponectin; venous blood; placenta; cows; calf birth weight

初生重是犊牛选育标准之一,对评估胎犊宫内发育状况及预测成年后生产性能有重要价值。胎儿宫内发育过程受不同因素,包括:母体营养状况、转录调节因子及脂肪因子(Adipokines)等的调控和影响,其中脂肪因子是重要调控因子之一^[1]。脂肪因子是由脂肪等组织分泌的活性物质,包括脂联素(Adiponectin)、瘦素(Leptin)、抵抗素(Resistin)、内脂素(Visfatin)、网膜素(Omentin)和肿瘤坏死因子 α (TNF- α)等 50 余种。脂联素是血液中含有量最高的脂肪因子,在糖、脂代谢调控网络中有重要作用,已有研究发现人和鼠静脉血、脐带血、胎盘组织和胎儿静脉血脂联素与胎儿初生重存在相关性^[2-6]。脂联素在奶牛血清、羊水、乳汁及脂肪等组织均有表达,并与奶牛发情、妊娠及犊牛生长发育密切相关^[7-9]。目前关于分娩奶牛静脉血、胎盘组织、脐静脉血及犊牛静脉血与犊牛宫内发育及犊牛初生重的相关性研究还未见报道。因此,本研究旨在探究奶牛静脉血、胎盘组织、脐静脉血及犊牛静脉血中脂联素的相关性及其与犊牛初生重的相关性,为进一步研究脂联素对胎犊宫内发育的作用机理提供参考。

1 材料与方法

1.1 试验动物

试验选择四川省某规模化奶牛场半封闭统一舍饲,体重 500 kg 左右,2~4 胎中国荷斯坦奶牛 95 头,从中选择体况良好、产前临床检查健康、妊娠足

月、自然分娩、脐带与胎盘正常及犊牛发育正常的奶牛 54 头。

1.2 试验材料

脂联素双抗体夹心酶联免疫吸附检测(ELISA)试剂盒,购自南京建成生物工程研究所,灵敏度 0.5 ng/mL,使用 ELx800 酶标仪(美国 BIO-TEK 公司)检测。

1.3 试验方法

1.3.1 血液收集

采集分娩奶牛颈静脉血 10 mL,分娩后立即采集脐静脉血、犊牛颈静脉血各 10 mL,置于未加抗凝剂的离心管中,室温静置 1 h,离心力 352 g 离心 10 min,收集上层血清, -20 °C 冻存,待检。

1.3.2 胎盘收集

奶牛分娩后立即手术法随机剥离子宫内子叶上胎盘组织 2 块,生理盐水冲洗 3 次,用滤纸吸干残余液体,放入样品袋并标记,液氮保存。

1.3.3 犊牛初生重测定及分组

犊牛出生后立即擦干羊水和血渍,测量新生犊牛初生重。其中犊牛初生重 ≤ 40 kg 的母牛记入 A 组(9 头);犊牛初生重 40~45 kg 的母牛记为 B 组(25 头);犊牛初生重 ≥ 45 kg 的母牛记为 C 组(20 头)。

1.3.4 ELISA 检测

从液氮中取出胎盘组织,液氮低温研磨,称量研磨胎盘组织 1 g,加入 10 mL 生理盐水稀释,混匀离

心后取上清待测。血清解冻后备用。按双抗体夹心酶联免疫吸附技术(ELISA)检测试剂盒说明书检测样品脂联素水平。

1.4 统计分析

统计学分析采用 SPSS 19.0 软件。K-S 检验计量资料是否服从正态分布,以 $\bar{X} \pm S$ 表示,组间差异采用独立样本 t 检验,多组间比较采用单因素方差分析,相关性分析采用双变量 Pearson 相关分析, $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 各组奶牛静脉血、脐静脉血、胎盘组织及犊牛静脉血脂联素水平

奶牛静脉血、脐静脉血、胎盘组织及犊牛静脉血脂联素水平见表 1,奶牛静脉血、胎盘组织、脐静脉

血及犊牛静脉血中均有脂联素表达。随犊牛初生重增加,奶牛静脉血脂联素水平呈逐渐下降趋势,但差异均不显著($P > 0.05$);随犊牛初生重的增加,胎盘组织、脐静脉血及犊牛静脉血脂联素水平逐渐升高,其中 C 组脐静脉血和犊牛静脉血脂联素水平显著高于 A 组($P < 0.05$),但 A 组和 B 组、B 组和 C 组间差异均不显著($P > 0.05$);C 组胎盘组织脂联素水平显著高于 A 组和 B 组($P < 0.05$),A 组和 B 组间差异不显著($P > 0.05$)。

2.2 奶牛静脉血、脐静脉血及犊牛静脉血脂联素水平

奶牛静脉血、脐静脉血及犊牛静脉血脂联素水平见表 2。从表 2 可知,奶牛静脉血脂联素水平极显著高于脐静脉血和犊牛静脉血脂联素水平($P < 0.01$),脐静脉血和犊牛静脉血中脂联素差异不显著($P > 0.05$)。

表 1 各组奶牛静脉血、脐静脉血、胎盘组织及犊牛静脉血脂联素水平

Table 1 Adiponectin levels in cow's venous blood, cord blood, placenta and calves' venous blood in each groups

分组 Group	样本数 No. of sample	初生重/kg Birth weight	脂联素 Adiponectin			
			奶牛静脉血/ (mg/L) Cow's venous blood	胎盘组织/ ($\mu\text{g/g}$) Placenta	脐静脉血/ (mg/L) Cord blood	犊牛静脉血/ (mg/L) Calves' venous blood
A	9	36.68 \pm 2.12 C	31.41 \pm 4.23 a	171.91 \pm 18.59 b	13.16 \pm 1.23 b	12.94 \pm 1.08 b
B	25	42.42 \pm 1.58 B	28.74 \pm 3.34 a	184.47 \pm 21.46 b	13.5 \pm 0.90 ab	13.36 \pm 0.78 ab
C	20	48.56 \pm 3.59 A	28.64 \pm 4.55 a	198.04 \pm 19.62 a	14.36 \pm 1.16 a	13.82 \pm 0.96 a

注:同列不同小写字母表示差异显著($P < 0.05$),不同大写字母表示差异极显著($P < 0.01$),有相同大、小写字母表示差异不显著($P > 0.05$)。下表同。

Note: Different lower case letters indicate significant differences ($P < 0.05$). Different upper case letters indicate highly significant differences ($P < 0.01$). Same lower case or upper case letters indicate no significant differences ($P > 0.05$). The same below.

表 2 奶牛静脉血、脐静脉血及犊牛静脉血脂联素水平

Table 2 Adiponectin levels in cow's venous blood, cord blood and calves' venous blood

分组 Group	样本数 No. of sample	脂联素/(mg/L) Adiponectin
奶牛静脉血	54	29.15 \pm 4.02 A
脐静脉血	54	13.79 \pm 1.14 B
犊牛静脉血	54	13.46 \pm 0.94 B

2.3 奶牛静脉血、胎盘组织、脐静脉血及犊牛静脉血脂联素水平与犊牛初生重相关性

奶牛静脉血、胎盘组织、脐静脉血及犊牛静脉血脂联素水平与犊牛初生重相关性如表 3 所示,犊牛初生重与奶牛静脉血脂联素水平相关性不显著

($P > 0.05$),与脐静脉血、胎盘组织脂联素水平极显著正相关($P < 0.01$),与犊牛静脉血脂联素水平显著正相关($P < 0.05$);奶牛静脉血脂联素水平与脐静脉血、胎盘组织和犊牛静脉血脂联素水平差异均不显著($P > 0.05$);脐静脉血脂联素水平与犊牛静

脉血极显著正相关($P < 0.01$),与胎盘组织脂联素水平相关性不显著($P > 0.05$);胎盘组织脂联素水

平与犊牛静脉血脂联素水平相关性不显著($P > 0.05$)。

表 3 奶牛静脉血、胎盘组织、脐静脉血、犊牛静脉血脂联素水平与犊牛初生重相关性

Table 3 Correlation between the calf birth weight and adiponectin levels in cow's venous blood, placenta, cord blood and calves' venous blood

指标 Index	奶牛静脉血 Cow's venous blood		胎盘组织 Placenta		脐静脉血 Cord blood		犊牛静脉血 Calves' venous blood	
	r 值	P 值	r 值	P 值	r 值	P 值	r 值	P 值
初生重	-0.86	0.538	0.365**	0.007	0.417**	0.002	0.314*	0.021
奶牛静脉血			-0.165	0.233	-0.043	0.757	-0.078	0.576
胎盘组织					0.094	0.498	0.062	0.654
脐静脉血							0.552**	<0.001

注:r:相关系数;*代表相关性显著($P < 0.05$);**代表相关性极显著($P < 0.01$)。

Note:r indicates correlation coefficient;* indicates significant difference ($P < 0.05$); ** indicates highly significant differences ($P < 0.01$).

2.4 奶牛静脉血、脐静脉血、胎盘组织及犊牛静脉血脂联素水平的性别差异

生产不同性别犊牛的奶牛静脉血、脐静脉血、胎盘组织及不同性别犊牛静脉血脂联素水平如

表 4,可见,公犊牛初生重极显著高于母犊牛($P < 0.01$),奶牛静脉血、脐静脉血、胎盘组织及犊牛静脉血脂联素水平在公、母犊间差异均不显著($P > 0.05$)。

表 4 生产不同性别犊牛的奶牛静脉血、脐静脉血、胎盘组织及不同性别犊牛静脉血中脂联素水平

Table 4 Adiponectin levels in cow's venous blood, cord blood, placenta and calves' venous blood of male and female calves

犊牛 Calves	样本数 No. of sample	初生重/kg Birth weight	脂联素 Adiponectin			
			奶牛静脉血/ (mg/L) Cow's venous blood	胎盘组织/ ($\mu\text{g/g}$) Placenta	脐静脉血/ (mg/L) Cord blood	犊牛静脉血/ (mg/L) Calves' venous blood
公 Male	22	46.09 \pm 5.30 A	29.11 \pm 4.69 a	191.00 \pm 24.46 a	13.87 \pm 1.28 a	13.54 \pm 1.01 a
母 Female	32	42.18 \pm 4.02 B	29.17 \pm 3.58 a	184.93 \pm 20.26 a	13.74 \pm 1.05 a	13.40 \pm 0.89 a

3 讨论

3.1 奶牛静脉血、胎盘组织、脐静脉血及犊牛静脉血脂联素水平与犊牛初生重的相关性

脂联素主要由脂肪组织分泌,是血液中含有最高的脂肪因子,近年研究发现,母体脂联素对胎儿宫内生长发育具有一定的影响。Kadowaki 等^[10]研究发现肥胖孕妇脂联素降低,易导致胎儿过大。Rosario 等^[11]发现小鼠母体脂联素水平下降后,胎鼠体重升高。Aye 等^[6]发现注射外源性脂联素小鼠胎鼠体重较未注射小鼠轻。但张婵等^[12]和王利玲

等^[13]指出母体脂联素水平与胎儿初生重相关性不显著。本研究结果显示,奶牛静脉血脂联素水平与犊牛初生重相关性也不显著($P > 0.05$),但随犊牛初生重增加,奶牛静脉血脂联素水平有下降趋势,与上述学者研究结果趋势一致,推测脂联素能调节母体糖和脂肪代谢,促进奶牛血浆中游离脂肪酸氧化,提高胰岛素敏感性,增加母体对葡萄糖的摄取,使宫内胎犊糖、脂供应减少,从而影响犊牛宫内生长发育,以防胎儿过大。胎盘脂联素在妊娠各阶段对胎儿发育影响不同。妊娠早期,胎盘脂联素促进滋养层细胞增殖及入侵,调节内皮细胞凋亡分化,参与绒

毛膜绒毛血管生成,促进胎盘形成^[14];妊娠中、晚期,胎盘脂联素通过影响葡萄糖转运蛋白-3 (GLUT3)、脂蛋白酶(LPL)、转化生长因子(TGF)等表达,调节母体葡萄糖和甘油三酯向胎儿转运,影响胎儿生长发育。脂联素能增加胎儿对胰岛素和胰岛素样生长因子的敏感性^[15],激活骨骼肌和肝脏中特异性受体,增加糖摄取,抑制糖异生,促进胎儿机体同化作用,增加成骨细胞活性,利于胎儿骨骼发育,从而促进胎儿增重;通过上调滋养层细胞表面的CD24和Siglec10,提高胎儿免疫耐受^[16],保证胎儿正常发育。但Dawczynski等^[17]发现人胎盘脂联素表达量低,且与胎儿初生重不相关,而王晶等^[5]发现胎儿初生重较高时,胎盘脂联素表达较低,胎盘脂联素与胎儿初生重呈负相关。本研究结果显示,奶牛胎盘组织能表达脂联素,且随犊牛初生重增加,脂联素水平逐渐升高,C组胎盘脂联素水平显著高于A组和B组($P < 0.05$),且胎盘脂联素水平均与犊牛初生重呈极显著正相关($P < 0.01$),与上述研究结果有差异,提示奶牛胎盘脂联素有利于犊牛宫内生长发育,推测胎盘脂联素通过上述机制促进胎盘的形成功能和发育,从而促进宫内胎犊生长发育。

脐静脉血脂联素与胎儿宫内发育的相关性存在一定争议。Cekmez等^[4]、王利玲等^[13]和孙妍等^[18]均发现胎儿脐血脂联素与新生儿初生重呈正相关,但Lindsay等^[19]发现胎儿脐血脂联素与胎儿初生重相关性不显著,Corbetta等^[20]也发现早产儿脐血脂联素水平与初生重无相关性,但随胎龄的延长而增加,是胎龄而不是初生重影响脐血脂联素水平。而Cekmez等^[21]研究结果则表明脐静脉血脂联素与初生重呈负相关。目前关于奶牛脐静脉血脂联素与犊牛初生重相关性的研究还未见报道,本研究结果显示,随犊牛初生重增加,脐静脉血脂联素水平有升高趋势,且与犊牛初生重呈极显著正相关($P < 0.01$),与Cekmez等^[4]、张妍等^[18]和孔丽君等^[22]研究人脐血脂联素与胎儿初生重呈正相关的结果相一致,推测奶牛脐静脉血脂联素能够促进胎儿宫内生长发育,其增加胎儿对胰岛素和IGF-1敏感性,激活骨骼肌和肝脏中特异性受体,增加糖摄取,抑制糖异生,促进胎儿增重。董红红等^[23]发现早产儿血清脂联素显著低于足月儿,且胎龄越大脂联素水平越高。Kesser等^[24]研究也发现早产犊牛血清脂联素低于足月产犊牛。本研究结果显示随犊牛体重增加,犊牛静脉血脂联素水平有升高趋势,且犊牛静脉血脂

联素水平与犊牛初生重呈显著正相关($P < 0.05$),与上述学者研究结果相似,提示犊牛自分泌的脂联素能促进犊牛宫内生长发育,影响犊牛初生重。

由于哺乳动物具有胎盘屏障,且胎盘有自分泌功能,导致母体和胎儿间处于相对独立又相互联系的状态。母体和胎儿通过胎盘内绒毛间隙进行物质交换,同时胎盘自分泌的因子进入脐静脉输送至胎儿。推测母体脂联素因属大分子物质不能通过胎盘组织,胎盘组织脂联素和胎儿脂联素可能均源于自分泌。Kesser等^[24]研究发现早产犊牛血清脂联素水平低于足月产犊牛,且均在饲喂初乳后显著升高,间接表明奶牛胎盘可能不转运脂联素。本研究结果显示,胎盘组织脂联素与奶牛静脉血、脐静脉血、犊牛静脉血脂联素相关性均不显著($P > 0.05$),奶牛静脉血脂联素极显著高于犊牛静脉血和脐静脉血($P < 0.01$),而犊牛静脉血和脐静脉血脂联素差异不显著($P > 0.05$),且两者呈极显著正相关($P < 0.01$),也证明了因胎盘存在屏障作用,脂联素难以通过胎盘屏障,奶牛母体脂联素、胎盘脂联素与宫内胎犊脂联素相互独立,母体脂联素能调节母体糖和脂肪代谢,而非直接影响胎犊;胎盘自分泌脂联素主要通过调节胎盘转运功能影响犊牛发育,对犊牛脂联素的影响较小,而犊牛自分泌的脂联素对犊牛宫内发育可能起主要调节作用^[6]。奶牛静脉血脂联素与犊牛初生重相关性不显著($P > 0.05$),而胎盘组织、脐静脉血脂联素与犊牛初生重极显著正相关($P < 0.01$),犊牛静脉血脂联素与犊牛初生重呈显著正相关($P < 0.05$),与田朝霞等^[25]研究结果一致,说明犊牛静脉血脂联素来自犊牛自身脂肪等组织,与母体脂联素无明显相关性,提示脐静脉血和犊牛静脉血均在同一循环系统,所以两者脂联素变化趋势一致,且相互呈正相关。另外,胎盘自分泌的脂联素通过脐静脉到达胎犊,利于胎犊宫内生长发育。

3.2 奶牛静脉血、脐静脉血、胎盘组织及犊牛静脉血脂联素水平的性别差异

有研究资料表明,在胎儿、儿童及成年阶段,脂联素水平均与性别有关^[26],一方面因为激素直接与脂联素作用,另一方面是因为不同性别机体脂肪量及分布不同,从而导致脂联素含量不同。新生儿性别与机体脂联素水平的关系尚不明确。Kotani等^[27]指出早产女性胎儿脂联素高于男性,孔丽君等^[22]发现足月女性胎儿脐静脉血脂联素显著高于男性,Troisi等^[28]研究发现妊娠中期,男胎睾酮较

女胎高5倍,但到足月时下降至少于1.5倍,推测雄激素能抑制脂联素表达;Ibanez等^[29]研究表明小于胎龄儿(SGA)组中女性胎儿脐静脉血脂联素高于男性胎儿,而适于胎龄儿(AGA)组未发现性别差异,有研究显示健康足月儿中脂联素不出现性别差异^[27],而本研究中奶牛静脉血、胎盘组织、脐静脉血及犊牛静脉血脂联素水平在不同性别犊牛中均差异不显著($P>0.05$),提示犊牛性别对奶牛静脉血、胎盘组织、脐静脉血及犊牛静脉血脂联素的影响较小,可能由于妊娠末期,公、母犊均从母体大量吸收营养,生长迅速,而使脂联素的性别差异减弱。

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