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論文名	Secretary pattern of insulin-like peptide 3 and its regulation in male ruminants （雄反芻動物におけるインスリン様ペプチド3の分泌パターンとその調節）	
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論文要旨

Introduction

Insulin-like peptide 3 (INSL3), along with testosterone, is a major secretory product of testicular Leydig cells in males. The important roles of INSL3 in male reproduction are to complete the trans-abdominal phase of testicular descent and play as an anti-apoptotic factor in germ cell survival.

Peripheral levels of INSL3 have been detected in males of many species, but there are no report yet with frequent blood sampling to investigate the secretory nature of INSL3 and its temporal relationships with luteinizing hormone (LH) and testosterone. In men, an increase in testosterone concentrations was observed with a single injection of human chorionic gonadotropin (hCG) which has an LH-like activity, but any changes in INSL3 secretion were not observed. Therefore, the regulation of INSL3 secretion was thought to be different from testosterone. However, the regulation of INSL3 secretion by stimulation or suppression of LH has not been studied in male domestic animals.

Scrotal circumference is a good measure of puberty, which has been discussed details in bulls. INSL3 has been suggested as a testis-specific biomarker for assessing pubertal development in several species. It was shown that the dynamics of the secretory patterns of INSL3 in bulls and male dogs are different during pubertal development. However, INSL3

profile during pubertal development and its association with scrotal circumference in small ruminants are yet to be elucidated.

A series of following in vivo studies was done to unveil the secretory pattern of INSL3 and its regulation in male ruminants.

Chapter 1: Secretory pattern and regulation of INSL3 in bulls

The objectives were to determine the temporal relationship of pulsatile secretion among LH, INSL3 and testosterone, and to monitor acute regulation of INSL3 secretion by LH using GnRH analogue and hCG in bulls. Blood samples at 15-min intervals revealed that the secretion of LH, INSL3 and testosterone in the blood is pulsatile with a frequency of 4.7 ± 0.9 , 3.8 ± 0.2 and 1.0 ± 0.0 , respectively, during the 8 h period. Seventy percent of these INSL3 pulses peaked within 1 h after a peak of an LH pulse had occurred. The mean increase (peak/basal concentration) of testosterone pulses was higher than those of INSL3 pulses. After GnRH treatment, LH concentrations increased dramatically 1 h later and remained high until the end of sampling at hour 6, whereas an elevated INSL3 concentration occurred at 1 and 2 h after the treatment. Testosterone concentrations increased 1 h after the treatment and remained high until the end of sampling. After hCG treatment, an increase of INSL3 concentration occurred at 2 and 4 h, and Days 2, 4 and 8 after the treatment, whereas in case of testosterone, concentrations remained high until Day 8 after the treatment. The increase (maximum/pre-treatment concentration) of INSL3 concentrations after injecting GnRH or hCG was much lower than those of testosterone. In conclusion, secretion of INSL3 occurred in a pulsatile manner in bulls. We inferred an acute regulation of INSL3 by LH in bulls because INSL3 concentrations increased immediately after endogenous and exogenous LH stimulation. The increase of INSL3 concentrations by LH was much lower than that of testosterone in bulls.

Chapter 2: Secretory pattern and regulation of INSL3 in male goats

In order to check whether or not the similar secretory pattern and regulation of INSL3 shown for bulls is existed in small ruminants, the regulation of plasma INSL3 secretions and its relationships with LH and testosterone were examined in male goats. After GnRH treatment, mean plasma concentrations of all 3 hormones increased dramatically from 30 minutes and remained high until 120 minutes (LH), 75 minutes (INSL3) and 4 hours (testosterone). After hCG treatment, mean plasma INSL3 concentrations increased from 30 minutes and remained elevated until the end of sampling on Day 12. An increase in mean plasma testosterone concentrations occurred from 15 minutes and remained high until Day 6. The mean increase of INSL3 concentrations after administration of GnRH and hCG was lower than that for testosterone. The secretory pattern of LH, INSL3 and testosterone in the general circulation was pulsatile with a frequency of 5.5 ± 0.6 , 4.7 ± 0.5 and 2.2 ± 0.5 , respectively, during the 8-hour period. Twenty out of 28 (71%) of these INSL3 pulses peaked within 1 hour after a peak of an LH pulse. The mean increase of INSL3 pulses was

lower than that of testosterone pulses. The scrotal circumference per body weight was 14.4-time and mean plasma INSL3 concentrations were 9.3-time higher in male goats than in bulls of chapter 1. The results indicated that the secretion of INSL3 in blood occurred, like in bulls, in a pulsatile manner soon after LH pulses in male goats. The absolute concentrations of INSL3 in male goats was higher than that reported in other mammals. INSL3 concentrations were acutely increased by endogenous and exogenous LH in male goats, but the rise of INSL3 was lower than that of testosterone.

Chapter 3: Effects of a long-acting GnRH antagonist on INSL3 and testosterone concentrations, and scrotal circumference in male goats

It is shown in above chapters that plasma INSL3 concentrations increased soon after stimulations of LH. However, the effects of LH suppression on INSL3 secretion are unknown in domestic animals. Here, the effects of a long-acting GnRH antagonist (degarelix acetate) on the secretions of plasma INSL3 and testosterone were studied in two phases. During the immediate phase, blood was taken at 15-minute intervals for 8 hours on Days -5, 0 and 3. The GnRH antagonist was administered after 2-hour sampling of Day 0. In the long-term phase, a daily blood sample was taken from Day 0 to 7, followed by twice weekly until 9 weeks and finally at week 10. The scrotal circumference was recorded before the treatment and continued biweekly until week 10. The mean concentrations, pulse frequency (per hour) and pulse amplitude (peak – nadir) of plasma LH and testosterone reduced from pre-treatment (combined Day -3 and Day 0) to post-treatment Day 0 and Day 3. A decline in mean concentrations, pulse frequency and pulse amplitude of INSL3 was found on post-treatment Day 3 compared to pre-treatment. During long-term sampling, a decline in plasma testosterone and INSL3 concentrations was observed 1 day after the treatment and remained lower until 8.5 weeks post-treatment, and returned to pre-treatment levels thereafter. A reduction in scrotal circumference was recorded 4 weeks after the treatment and remained lower until 10 weeks post-treatment. In conclusion, the acute regulation of INSL3 by LH was confirmed by reduction of plasma INSL3 levels after GnRH antagonist treatment. The lower levels of INSL3 and testosterone persisted for 8.5 weeks with subsequently returned to pre-treatment levels. A significant reduction in testicular size was also observed. GnRH antagonist could be used for reversible long-term chemical castration in male goats.

Chapter 4: Changes of INSL3 and testosterone and their association with scrotal circumference during pubertal development in male goats

The objectives were to determine age-related changes of plasma concentrations of INSL3 and testosterone, and their association with scrotal circumference during pubertal development in male goats. Blood sampling and measurement of scrotal circumference were done biweekly from week 14 to week 52. Based on changes of the scrotal circumference, data were grouped into early, late and post-pubertal categories. Plasma

INSL3 concentrations increased continuously during and after puberty, whereas plasma testosterone concentrations were fluctuated during the same periods. The R^2 value of best regression curves between scrotal circumference and INSL3 was higher than between scrotal circumference and testosterone. The results suggested that plasma INSL3 concentrations increased continuously during and after puberty, whereas testosterone secretions were fluctuated in male goats. The scrotal circumference was more highly correlated with the INSL3 concentrations than with testosterone, implying that INSL3 is superior as a biomarker of testicular total Leydig cell volume.

Overall conclusions

1. The secretion of INSL3 in the general circulation of bulls and male goats was pulsatile.
2. INSL3 secretion was acutely regulated by LH in bulls and male goats.
3. The increase of plasma INSL3 concentrations during normal pulsatile secretion and after LH stimulation was much lower than that for testosterone in bulls and male goats.
4. The absolute concentration of INSL3 in male goats was much higher than that in bulls.
5. The regulation of INSL3 by LH was further confirmed by a long-acting GnRH antagonist treatment in male goats.
6. GnRH antagonist can be used for reversible long-term chemical castration in male goats.
7. Scrotal circumference was more strongly correlated with plasma INSL3 concentrations than with testosterone concentrations during pubertal development in goats. Thus, INSL3 is suggested superior as a biomarker of testicular total Leydig cell volume in male goats.

審査結果の要旨

インスリン様ペプチド3 (INSL3) は動物の精巣ライディッヒ細胞から分泌されるペプチドホルモンであり、同細胞から分泌されるテストステロンとともに、胎子期の精巣下降および性成熟後の精子形成において重要な役割を果たすことがげっ歯類で報告されている。男性における INSL3 の分泌は黄体形成ホルモン (LH) の長期的な作用によって促進されるが、短時間での血中濃度の変動はなく、LH による即時的な調節を受けないとされており、テストステロンの分泌が LH の即時的な調節を受けて増減することとは対照的であると報告されている。しかし、ヒト以外の動物、特に反芻動物における血中 INSL3 濃度の短時間内での変動とその調節因子については全く不明である。さらに雄反芻動物において性

成熟の指標となる陰嚢周囲長（精巣容積を表す）と血中 INSL3 濃度との関連性を反芻動物で調べた報告はみあたらない。本研究は雄ウシと雄ヤギの LH による INSL3 の分泌制御と雄ヤギの性成熟過程における INSL3 分泌に焦点を絞って調べている。

第 1 章では、雄ウシにおける INSL3 を含めた性ホルモンの血中濃度の経時的変動を解析するとともに、内因性および外因性 LH による INSL3 分泌促進の有無を調べた。その結果、末梢血液への INSL3 分泌は LH によって即時的に調節されている拍動性分泌であり、LH 刺激後の血中 INSL3 濃度の増加率はテストステロンと比べると低いことを明らかにした。

第 2 章では、雄ヤギにおける INSL3 を含めた性ホルモンの血中濃度の経時的変動を解析するとともに、内因性および外因性 LH による INSL3 分泌促進の有無を調べた。その結果、雄ウシと同様に雄ヤギの末梢血液への INSL3 分泌は LH によって即時的に調節されている拍動性分泌であり、LH 刺激後の血中 INSL3 濃度の増加率はテストステロンと比べると低いことを明らかにした。また、雄ヤギの血中 INSL3 濃度は雄ウシと比べると 9 倍以上の高濃度で認められ、雄ヤギの体重に対する陰嚢周囲長の比率が雄ウシと比べると 14 倍以上であることと関連することを示唆した。

第 3 章では、雄ヤギを用いて LH の分泌を抑制する性腺刺激ホルモン放出ホルモン（GnRH）拮抗剤の INSL3 を含めた性ホルモンの血中濃度に及ぼす影響を調べた。その結果、GnRH 拮抗剤は INSL3 とテストステロンの分泌を約 8.5 週間抑制し、その後処置前のレベルに復帰することを示した。また、GnRH 拮抗剤の処置による精巣周囲長の減少を確認している。

第 4 章では、雄ヤギの性成熟過程における INSL3 を含めた性ホルモンの血中濃度と陰嚢周囲長との関連性を検討した。その結果、血中 INSL3 濃度は性成熟過程において持続的に増加したが、血中テストステロン濃度は変動が大きいことを示し、陰嚢周囲長は血中 INSL3 濃度と強く相関することを明らかにした。

以上の研究は、雄ウシと雄ヤギにおける短時間内の血中 INSL3 動態と LH による分泌調節を初めて明らかにし、血中 INSL3 濃度は従来から精巣ライディッシュ細胞の機能的指標として用いられているテストステロンよりも短時間内の変動の振幅が小さく、安定した指標になりうることを示した。また雄ヤギの血中 INSL3 濃度は陰嚢周囲長との相関性にも優れており、雄性生殖能の新たな指標として活用できる可能性が示唆された。さらに持続性 GnRH 拮抗剤は反芻動物の長期の可逆的去勢に活用しうることを示された。これらの研究

成果は、雄反芻動物の精巣内分泌機能の調節機構の解明、精巣機能と生殖能力の新たな判定法および人為調節法の開発に寄与すると考えられ、獣医繁殖学領域における生殖内分泌学の発展に資するものであり、本論文の審査ならびに最終試験の結果と併せて、博士（獣医学）の学位を授与することを適当と認める。