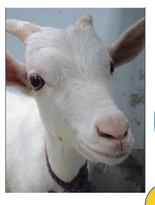
Establishment of immunoassay of new testicular hormone, insulin-like peptide 3, and its application



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MUSC Medical University of South Carolina Changing What's Possible

兵庫県 Hyogo Prefecture

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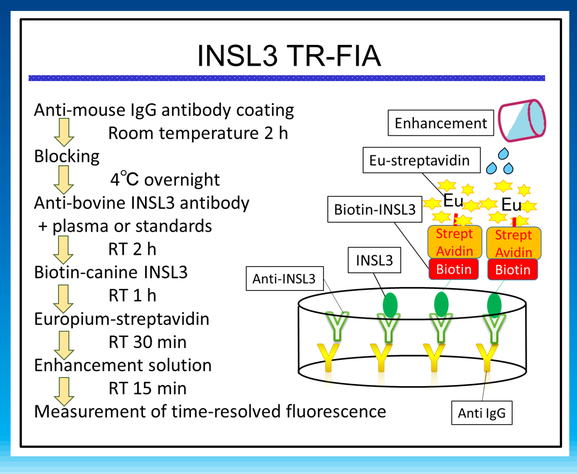
Collaborative research of Osaka Prefecture University (Lab of Theriogenology) with Medical University of South Carolina, Northern Center of Agricultural Technology in Hyogo Prefecture, Hyogo Prefecture College of Agriculture and Research Institute of Environment, Agriculture and Fisheries in Osaka Prefecture

Background & Objectives

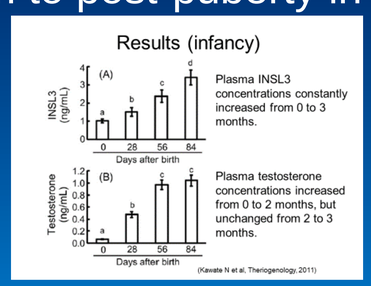
- Insulin-like peptide 3（INSL3）, which was discovered in 1993, is a hormone secreted from testicular Leydig cells.
- INSL3 has roles to stimulate testicular descent and spermatogenesis in mouse and rats.
- Secretory patterns and roles of INSL3 were unknown in farm and companion animals.
- INSL3 assays for cattle, goats and dogs were unavailable.
- Thus, we developed the INSL3 immunoassay procedures for the domestic animals.
- We are elucidating secretary dynamics of INSL3 in peripheral blood and examining applications to diagnose testicular functions.

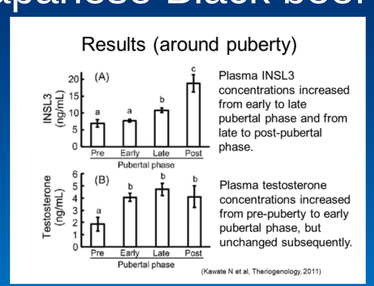
Establishment of INSL3 assay

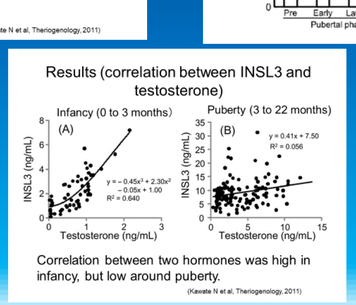
INSL3 EIA･TR-FIA protocol



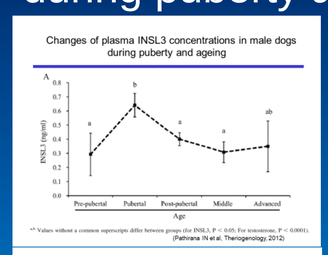
Changes of blood INSL3 concentrations from birth to post-puberty in Japanese Black beef bulls

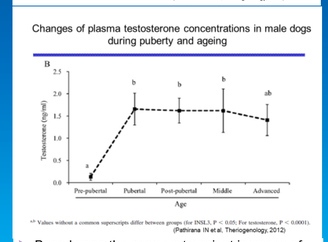






Plasma INSL3 concentrations in male dogs during puberty and with cryptorchid testis





- Based upon the assay, a transient increase of INSL3 was detected at pubertal age but it was not observed for testosterone, suggesting differing secretions of two hormones during puberty in male dogs.

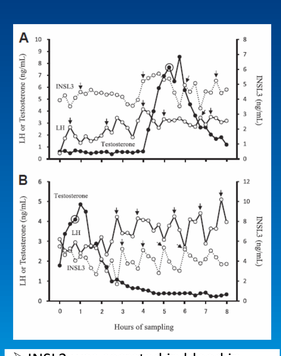
Comparison between normal and cryptorchid dogs of plasma INSL3 and testosterone concentrations

	Normal (n = 80)	UCO (n = 31)	BCO (n = 7)	Castrated (n = 3)
INSL3	0.49 ± 0.04	0.55 ± 0.08	0.21 ± 0.05[a]	UD
Testosterone	1.64 ± 0.21	1.57 ± 0.32	0.37 ± 0.10[a]	UD

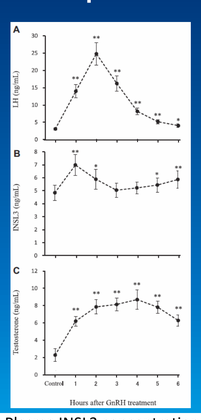
UD, undetectable; hormone concentration was below the minimum detection limit (INSL3<0.02 ng/ml; testosterone<0.04 ng/ml) of the assay.
[a]P<0.0001 compared with normal and unilateral cryptorchid dogs.
(Pathirana IN et al, Theriogenology, 2012)

- There seem to be impaired endocrine functions of Leydig cell components for paired retained testis in canine bilateral cryptorchidism.
- We suggest the diagnostic value of plasma INSL3 and testosterone concentrations for predicting bilateral retained testis in dogs.

Short-time dynamics of blood INSL3 concentrations and its regulation in Japanese Black beef bulls

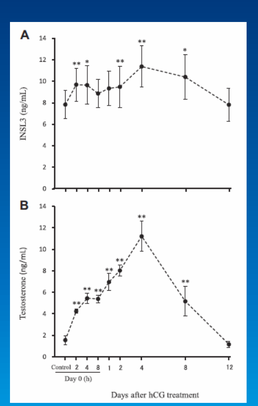


- INSL3 was secreted in blood in pulsatile manners and most of the pulses were followed by LH pulses.



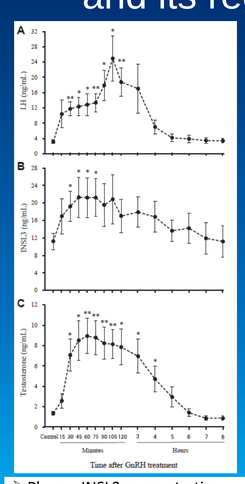
- Plasma INSL3 concentrations increased transiently after GnRH treatment, but its rise was much smaller than that of testosterone.

(Hannan MA et al, Therigenology, 2015)

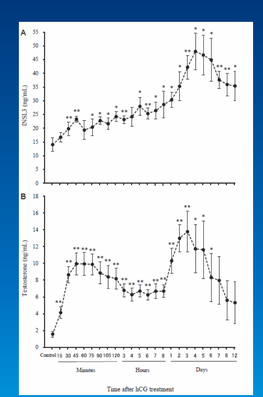


- Plasma INSL3 concentrations increased after hCG (LH) treatment, but its rise was much smaller than that of testosterone.

Short-time dynamics of blood INSL3 concentrations and its regulation in Shiba male goats

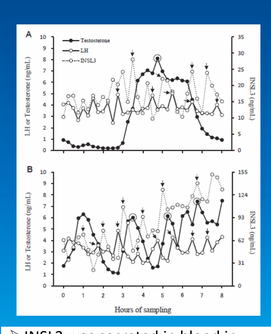


- Plasma INSL3 concentrations increased transiently after GnRH treatment, but its rise was smaller than that of testosterone.



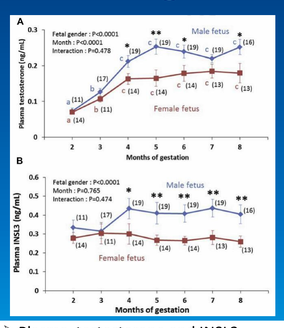
- Plasma INSL3 concentrations increased after hCG (LH) treatment, but its rise was much smaller than that of testosterone.

(Hannan MA et al, Therigenology, 2016)



- INSL3 was secreted in blood in pulsatile manners and most of the pulses were followed by LH pulses.

Fetal gender prediction based on maternal plasma testosterone and INSL3 concentrations at midgestation and late gestation in cattle



- Plasma testosterone and INSL3 concentrations were higher for dams (Japanese Black beef) with a male fetus at 4 to 6 mo and 8 mo than those with a female fetus.

Table 3. Predictive values, detection rates and accuracy of fetal gender predicted based on maternal plasma testosterone and INSL3 concentrations in Japanese Black beef cows at mid- and late gestation

	Testosterone			INSL3	
	5 mo	6 mo	8 mo	4 mo	8 mo
Cutoff value (ng/mL) [a]	0.172	0.188	0.176	0.241	0.264
Predictive value [b]					
Male fetus	77.3% (17/22)	76.2% (16/21)	75.0% (15/20)	76.5% (13/17)	78.6% (11/14)
Female fetus	81.8% (9/11)	75.0% (9/12)	88.9% (8/9)	62.5% (10/16)	66.7% (10/15)
Detection rate [c]					
Male fetus	89.5% (17/19)	84.2% (16/19)	93.8% (15/16)	68.4% (13/19)	68.8% (11/16)
Female fetus	64.3% (9/14)	64.3% (9/14)	61.5% (8/13)	71.4% (10/14)	76.9% (10/13)
Accuracy [d]	78.8% (26/33)	75.8% (25/33)	79.3% (23/29)	71.0% (23/33)	72.4% (21/29)

- The accuracy of fetal gender prediction by maternal testosterone at a single time point was nearly 80% from midgestation to late gestation while the accuracy by INSL3 was about 70% in the beef cows.

(Kibushi M et al, Therigenology, 2016)

Table 4. Predictive values, detection rates and accuracy of fetal gender predicted based on multiple time points (months) of maternal plasma testosterone and INSL3 concentrations in Holstein and Japanese Black beef breeds

	Holstein: Testosterone at 5 and 7 mo & INSL3 at 6 mo [a]	Japanese Black: Testosterone at 5 and 6 mo & INSL3 at 4 and 8 mo [b]	Japanese Black: Testosterone at 5, 6 and 8 mo & INSL3 at 4 mo [b]
Predictive value			
Male fetus [c]	85.7% (12/14)	87.5% (14/16)	80.0% (16/20)
Female fetus	92.9% (13/14)	88.9% (8/9)	100.0% (8/8)
Accuracy	89.3% (25/28)	88.0% (22/25)	85.7% (24/28)
Detection rate [d]			
Male fetus	75.0% (12/16)	73.7% (14/19)	84.2% (16/19)
Female fetus	86.7% (13/15)	57.1% (8/14)	57.1% (8/14)
Accuracy	80.6% (25/31)	66.7% (22/33)	72.7% (24/33)

- Nearly 90% accuracy for the prediction was obtained when multiple time points of testosterone and INSL3 concentrations from midgestation to late gestation.

Publications

Publications in academic journals（INSL3）

1. Hannan MA, Kawate N, Fukami Y, Weerakoon WW, Büllesbach EE, Inaba T, Tamada H. Changes of plasma concentrations of insulin-like peptide 3 and testosterone, and their association with scrotal circumference during pubertal development in male goats. Theriogenology. 2017;92:51-56.
2. Kibushi M, Kawate N, Kaminogo Y, Hannan MA, Weerakoon WWPN, Sakase M, Fukushima M, Seyama T, Inaba T, Tamada H. Fetal gender prediction based on maternal plasma testosterone and insulin-like peptide 3 concentrations at mid- and late gestation in cattle. Theriogenology. 2016;86(7):1764-1773.
3. Hannan MA, Kawate N, Fukami Y, Pathirana IN, Büllesbach EE, Inaba T, Tamada H. Acute regulation of plasma insulin-like peptide 3 concentrations by luteinizing hormone in male goats. Theriogenology. 2016;86(3):749-56.
4. Hannan MA, Fukami Y, Kawate N, Sakase M, Fukushima M, Pathirana IN, Büllesbach EE, Inaba T, Tamada H. Plasma insulin-like peptide 3 concentrations are acutely regulated by luteinizing hormone in pubertal Japanese Black beef bulls. Theriogenology. 2015;84(9):1530-5.
5. Hannan MA, Kawate N, Kubo Y, Pathirana IN, Büllesbach EE, Hatoya S, Inaba T, Takahashi M, Tamada H. Expression analyses of insulin-like peptide 3, RXFP2, LH receptor, and 3β-hydroxysteroid dehydrogenase in testes of normal and cryptorchid dogs. Theriogenology. 2015;84(7):1176-84.
6. Pathirana IN, Kawate N, Büllesbach EE, Takahashi M, Hatoya S, Inaba T, Tamada H. Insulin-like peptide 3 stimulates testosterone secretion in mouse Leydig cells via cAMP pathway. Regul Pept. 2012;178(1-3):102-6.
7. Pathirana IN, Yamasaki H, Kawate N, Tsuji M, Büllesbach EE, Takahashi M, Hatoya S, Inaba T, Tamada H. Plasma insulin-like peptide 3 and testosterone concentrations in male dogs: changes with age and effects of cryptorchidism. Theriogenology. 2012;77(3):550-7.
8. Kawate N, Ohnari A, Pathirana IN, Sakase M, Büllesbach EE, Takahashi M, Inaba T, Tamada H. Changes in plasma concentrations of insulin-like peptide 3 and testosterone from birth to pubertal age in beef bulls. Theriogenology. 2011;76(9):1632-8.
9. Pathirana IN, Kawate N, Tsuji M, Takahashi M, Hatoya S, Inaba T, Tamada H. In vitro effects of estradiol-17β, monobutyl phthalate and mono-(2-ethylhexyl) phthalate on the | secretion of testosterone and insulin-like peptide 3 by interstitial cells of scrotal and retained testes in dogs. Theriogenology. 2011;76(7):1227-33.
10. Pathirana IN, Ashida Y, Kawate N, Tanaka K, Tsuji M, Takahashi M, Hatoya S, Inaba T, Tamada H. Comparison of testosterone and insulin-like peptide 3 secretions in response to human chorionic gonadotropin in cultured interstitial cells from scrotal and retained testes in dogs. Anim Reprod Sci. 2011;124(1-2):138-44.

PhD student undergraduate student