Sugar Residues and Their Variations of Distribution on Ovarian Follicles of Timor Deer (*Cervus timorensis*)

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Abstract

Timor deer is one of Indonesian tropic deer has not most which investigated in Indonesia on biology of reproduction. The data of sugar residues containing on ovarian follicle of timor deer are important to support application of reproduction technology as effort and to increase timor deer utility as livestock with maximal quality.Ovaries isolated from 2 timor deers were fixed with bouin's and embedded in paraffin wax. Sections 5 µm, were deparaffined, rehydrated and labelled with four different lectins (PHA, RCA, Con A, and LCA). Preantral follicle has founded carbohydrate with D-N-acetylgalactosamine, galactose and mannose sugar residues in cytoplasm and zona pellucida oocyte, granulosa and teca cells. The antral follicle has not founded mannose in oocyte cytoplasm. D-N-acetylgalactosamine, galactose, mannose and glucose sugar residues have founded in zona pellucida, folicular liquid, granulosa and teca cells.

Key words : sugar residues, follicle,Timor deer (Cervus timorensis), lectin

Introduction

Timor deer is one of endemic ruminant. We have four kinds of tropic deer in Indonesia, they are Sambar, Timor, Muncak and Bawean deer. The Timor deer is prospective animal to be developed as source of animal protein low cholesterol with side products that has high economic values such as velvet, skin, tail, and testis (Semiadi and Nughraha 2004).

To develop new source of animal protein by consuming animals that have high potential of meal productivity called as prospective animal, based on ministry of agriculture's decree No.362/KPTS/TN. 12/5/1990 renewed by no. 404/KPTS/OT. 210/6/2002, deer have been officially categorized as livestocks (Semiadi and Nugraha 2004).

Ex situ reproduction of deer by captivity and application of reproduction technology is very important. Artificial insemination, IVF, ICSI are technology of reproduction that have been developed for other ruminants. The data of reproduction biology are important for developing technology of reproduction. The success in captivity and application of reproduction technology must be supported by researches on the tropical deer reproduction biology either in male or female. The research of Timor deer has not been conducted in Indonesia.

The ovary is divided into the outer cortex and central medulla regions. The medulla is composed mainly of blood vessels, lymphatics, connective tissue and nerves. The cortex is in perifer arround of medulla and contains follicles (Ross *et al*. 1995). During oestrus phase, follicles will develop (folliculogenesis) with different structure components.

Sugar residues are found in all follicle development, because they are sources of energy needed for follicle ovarian development. Besides, specific sugar residues on follicle are possible as keys in specific species of gamet interaction, gamet binding, and spermatozoon penetration during fertilization (Skutelsky E, et. al, 1994).

The research on sugar residues and their variations of distribution on ovarian follicle of Timor deer must be conducted to know the component and distribution of sugar residues that play role on follicle development. Therefore, it can be data for future application of reproduction technology.

Material and Method

Materials

In this experiment, ovaries were removed from mature deers (3-4 years old). The ovaries were washed with NaCl fisiologycal solution in 30-35°C temperature. The ovaries were fixed with bouin's solution and embedded in paraffin wax.

Histochemical analysis.

The paraffin wax embedded tissue sections (5 µm thick) were incubated the slide in 60^0C temperature for 30 minutes. So its were deparaffined with xylol and rehydrated with a series alcohol. The slides were washed with PBS and than were transfered to peroxide hydrogen (H_2O_2) 0,3% in PBS for inhibiting endogen peroxidase enzym activity. The slides were washed in running wáter and PBS solution 0,01 M. So It were incubated with peroxidase labeled lectin (Table 1) for 2 hours in 37 ^{0}C temperature. And than were washed with PBS to clean the no binding lectin. Visualisation were be done with 0.05% DAB (diaminobenzidine) and 0.3% H_2O_2 in tris buffer 0.05 M, pH 7.6. Contrast staining in nucleus has be used haematoxylin. Positive reactivity of lectin in the tissue has be showed in brown colour. To know specific reactivity, on all of histochemistry staining prosedure have be used positive and negative controls.

Table 1. Kinds, source, specific sugar and dosis in this research.

Lektin	**source**	**SpecificSugar**	**Dosis (µg/ml)**
PHA	*Phaseolus vulgaris*	GalNAc	5
RCA	*Ricinus communis*	Gal, GalNAc α	5
Con A	*Canavalia ensiformis*	Man α, Glc α	10
LCA	*Lens culinaris*	Man α	5

PHA = Phaseolus Vulgaris Erythroagglutinin, RCA = Ricinus Communis Agglutinin, Con A = Concanavalin A, LCA = Lens culinaris Agglutinin. GalNAc = D-N-Acetylgalactosamine, Gal = Galactose, Man α = mannose, Glc α = glucose.

The research observed the kind of carbohydrates and their distribution on ovarian follicle. The observation on affinity and intensity of lectin positive reaction in ovarian follicle have done at 2 slide for each lectin. The result of affinity and intensity have been clastered into 5 groups. There are strength (+++), medium (++), weak (+), very weak (+/-) and negative/ have not reaction (-). Observation and documentation the result used the camera microscope (Nikon Eclips, Tokyo, Japan).

Results and Discussion

The result of lectin hystochemistry's staining show the distribution of lectin binding with PHA, LCA, RCA dan ConA reaction variated in different follicle regions(Table 2). RCA lectin is specific for carbohydrate with galactose sugar residues. RCA positive reactions occur on all follicle regions with very weak to strong intensity (Picture 1A) and on all follicle development. Those are possible as playing role of galactose on all follicle development (preantral and antral phase). RCA has weak reaction in zona pellucida of antral follicle phase (Picture 2A) but In preantral follicle has positive reaction in medium into strong intensity. In buffalo ovary, zona pellucida's antral follicle have founded galactose residues (Parillo *et al.*

1998). According to Skutelsky *et al.* (1994), β-Galactose, D-N-acetylgalactosamine dan N-acetylglucosamine are common in rodent zona pellucida. Negative reaction of RCA Lectin has recorded in dog zona pellucida (Skutelsky *et al.* 1994). In granulosa cells of antral follicle phase, RCA intensity decreases into medium as the follicle development. Therefore, RCA intencity in follicular fluid increase in last follicle development. Positive reaction in granulosa cells and follicular fluids have recorded in most mammalia such as mouse, rat, hamster, rabbit, cat, dog and pig (Skutelsky *et al.* 1994).

Table 2. Variation and distribution of sugar residues on ovarian follicles of timor deer, *C. timorensis*

Lektin	**Part of follicle**	**Tipe follicle**	
		Preantral	**Antral**
PHA	externa teca	±	+
	interna teca	+	+
	Granulosa cells	±	+++
	follicular fluid	Not formed yet	+++
	Zona pellucida	++	-
	Oocyte cytoplasm	++	++
RCA	externa teca	++	+
	interna teca	++	+
	Granulosa cells	++~+++	++
	follicular fluid	Not formed yet	+++
	Zona pellucida	++~+++	±
	Oocyte cytoplasm	+~+++	+
LCA	externa teca	+	++
	interna teca	+	++
	Granulosa cells	+++	+++
	follicular fluid	Not formed yet	+++
	Zona pellucida	±	-
	Oocyte cytoplasm	+	-
Con A	externa teca	-	±
	interna teca	-	±
	Granulosa cells	-	-
	follicular fluid	Not formed yet	±
	Zona pellucida	±	-
	Oocyte cytoplasm	-	±

- = negative, ± = very weak, + = weak, ++ = medium, +++ = strong

PHA lectin binding indicate carbohydrate present with D-N-acetylgalactosamine residue. The results show that PHA lectin react positively with very weak into weak intensity in teca cells of preantral (picture 1B) and antral follicles. Increased intensity from very weak into strong occur in granulosa cells of last antral follicle development. Strong intensity occur in follicular fluids region too. The results so far show that carbohydrate with D-N-acetylgalactosamine residue probable as play the role on granulosa cells development and follicular fluids as granulosa cells secrete, in antral follicle phase. Medium reaction have been showed in zona pellucida and oocyte cytoplasm (picture 2 B). The carbohydrate with D-N-acetylgalactosamine residue has founded on all development phases. The carbohydrate with Beta-N-acetylgalactosamine residue have founded in early antral follicle development zona pellucida of buffalo ovary. (Parillo *et al.* 1998).

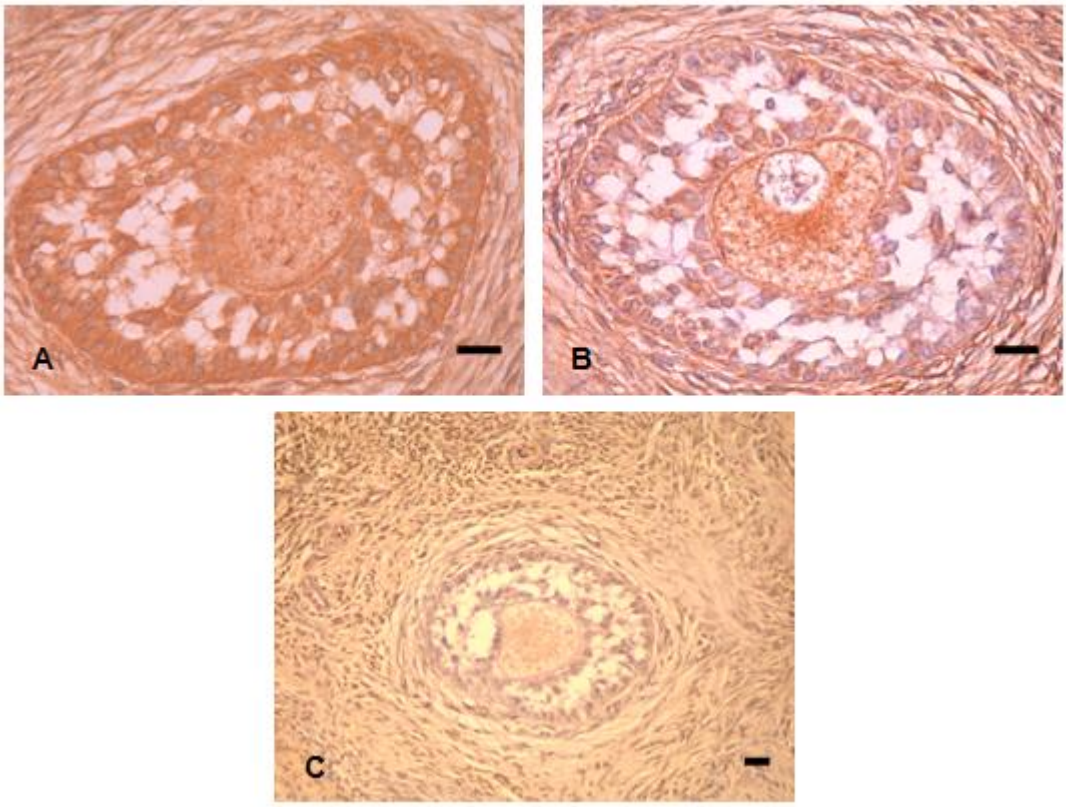
LCA lectin that indicates the carbohydrate with mannose residue show positive reaction in weak into medium intensity on interna and externa teca cells, (Picture 1C). Strong positive reaction have been seen in granulosa cells and follicular fluid. But positive reaction of LCA lectin with very weak intensity occur in zona pellucida and negative into very weak intensity in cytoplasm (Picture 2 C).

Mannose have not founded on antral follicle oocyte (zona pellucida and cytoplasm) as has recorded in cat follicle (Skutelsky *et al*. 1994) but have detected in all regions of preantral follicles (Picture 1 C). The results show that many mannose residues have founded in granulosa cells and follicular fluids. This results similar to recording in other mammal (Skutelsky *et al.*1994) and in early antral follicle of buffalo (Parillo *et al.* 1998).

Con A is the lectin detected carbohydrate with mannose and glucose residues in tissues. This results showed that have not founded mannose and glucose residues in some regions of Timor deer ovarían follicles as granulosa cells and zona pellucida of antral follicle (Picture 2 D). While this sugar residu have founded on all follicle development phase of mouse deer (Hamny *et.al.* 2008). No presented of mannose and glucose residues have also recorded in cat, dog and pig zona pellucida (Skutelsky *et al.* 1994).

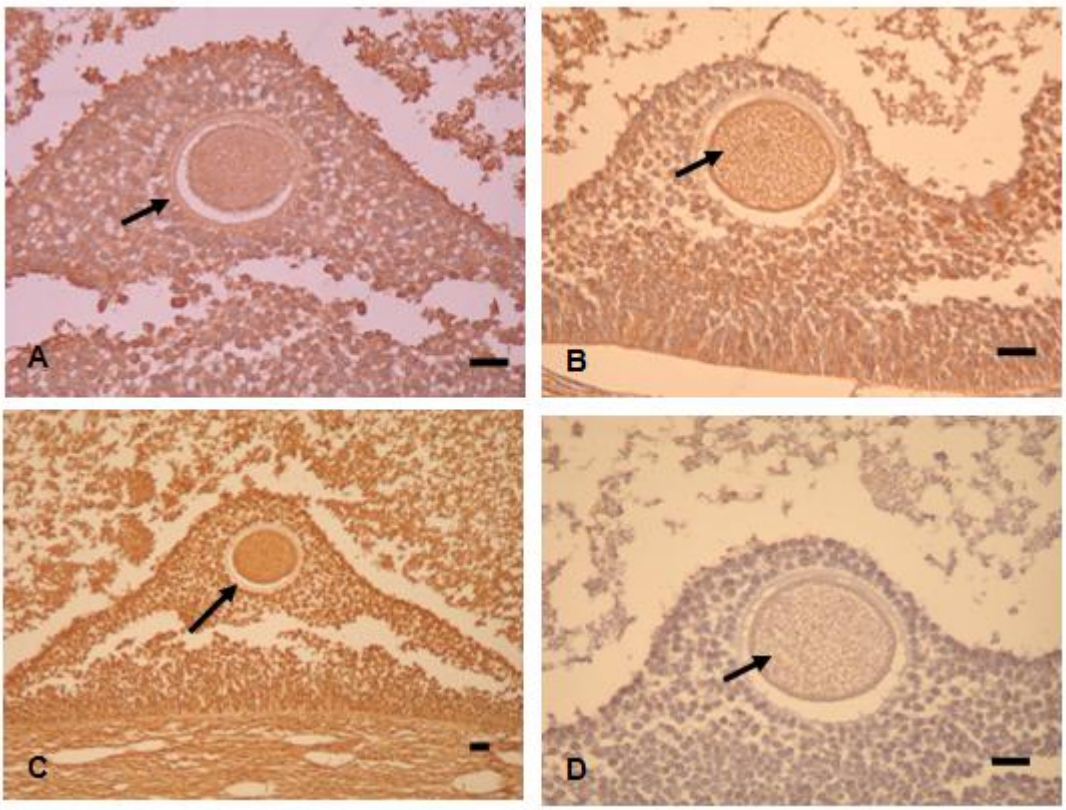
In lutein cells, PHA, LCA and RCA lectin show weak reaction, and negative reaction in Con A lectin. The positive reaction show that lutein cells contain carbohydrate with mannose, galactose dan D-N-acetylgalactosamine residues. In this research also have observed that interstitial cells arrounded follicle in ovarium contain D-N-acetylgalactosamine, galactose, mannose and any glucose residues.

Skutelsky *et al*. (1994) recorded that oocyte zona pellucida from different mammal species either show different lectin binding patterns. Sugar residues in zona pellucida are possible to play the role as spermatozoon reseptor. Carbohydrate with α-galactosyl plays the role on rat zona pellucida but in mouse, L-fucose, D-mannose and methyl mannosida have partisipated in spermatozoon binding. But not in hamster, rabbit and human; that partisipated are L-fucose and D-galactose. Antral follicle zona pellucida of Timor deer ovary have positive reaction of RCA lectin. This result demostrated that carbohydrate with galactose residues possible as play role in fertilization and in species specific of Timor deer oocyte zona pellucida.



Picture 1 Distribution lectin binding (brown) in preantral follicle. A. positive binding of RCA Lectin on all regions of follicle except on granulosa cells. B. positive binding of PHA Lectin with medium intencity on zona pellucida and oocyte cytoplasm, and weak reaction on granulosa cell matrix and teca cell. C. Positive binding of Lectin LCA on all regions of follicle. DAB, Bar 20 µm.

In follicular fluid of Timor deer have been founded any RCA lectin reaction that demostrated presented of glucose but have been founded many D-N-acetylgalactosamine, galactose dan mannose residues. Hafez and Hafez (2000) said that component and metabolite of follicular fluid were glucose, fructose, fucose, galactose, mannose, glucosamine, galactosamine, hyaluronat acid, heparin and plasminogen. There were assumted that component in follicular fluid will be different in other species. Follicular fluid of buffalo ovarian follicle contains glicoconjugate with beta-N-acetylgalactosamine, beta-galactose-(1-3)-N-acetylgalactosamine, beta-galactose-(1-4)-N-acetylglucosamine, N-acetylglucosamine, alfa-fucose, alfa-glucose, alfa-mannose and cyalic acid (Parillo *et al.* 1998).



Picture 2. Distribution of lectin binding on last phase of antral follicle. A. very weak positive reaction of RCA Lectin on zona pellucida (arrow). B. positive reaction of Lectin PHA with medium intencity on oocyte cytoplasm (arrow) and granulosa cell matrix. C. negative reaction of lectin LCA on zona pellucida (arrow). D. very weak positive reaction of lectin Con A on oocyte citoplasma (arrow). DAB, Bar 20 µm.

In oocyte cytoplasm of preantral follicle showed positive reaction of lectins that indicated present of carbohydrate with D-N-acetylgalactosamine, galactose, mannose dan glucose residues. But in antral follicle oocyte cytoplasm, mannose residues have not founded. This component dynamica indicate development proccess in oocyte cytoplasm.

CONCLUSION

1. Ovarian follicles of Timor deer contain carbohydrate with sugar residues D-N-acetylgalactosamine, galactose, mannose and glucose with intencity and variation of distribution on follicle development.
2. In early development (preantral follicle), oocyte cytoplasma and zona pellucida, granullosa cells and teca cells contain D-N-acetylgalactosamine, galactose and mannose.
3. The antral follicle has not founded mannose sugar residu on oocyte citoplasma and zona pellucida.
4. In antral follicle fase, oocyte citoplasma contain D-N-acetylgalactosamine dan galactose. zona pellucida contains low galactose. Carbohydrate with sugar residues D-N-acetylgalactosamine, galactose, mannose dan glucose have be founded on follicular fluids, granulosa and teca cells with different intencity.

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