UNIVERSITI PUTRA MALAYSIA

INFLUENCE OF HEPARIN AND SEMINAL HEPARIN BINDING PROTEINS ON REPRODUCTIVE PERFORMANCE OF SAHIWAL-FRIESIAN BULLS AND RABBITS

RAMAKRISHNAN A/L PATCHAIAPPAN

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

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Chairman: **Prof. Dato' Dr. Sheikh Omar Abdul Rahman**

Faculty: **Faculty of Veterinary Medicine**

A study was undertaken to characterise seminal heparin binding proteins (HBP) of Sahiwal-Friesian bulls and rabbits and to investigate the influence of HBP on the reproductive performance of Sahiwal-Friesian bulls and rabbits.

Using one dimensional sodium dodecyl sulfate-polyacrylamide (SDS-PAGE) gel electrophoresis, the major classes of HBP identified and isolated from Sahiwal-Friesian bulls and rabbits were with molecular weights of 15, 25, 30, 38 and 42, 15, 25, 38, 42 and 101 kDa, respectively. Bulls of varying fertility were found to have similar classes of HBP in their seminal fluids in the present study. This may show that the presence of the different classes of HBP in seminal fluid may not be an indicative of bull fertility.

Heparin and lyophilised HBP were found to have no effect on the percentage of spermatozoa motility in Sahiwal-Friesian bulls and rabbits. However, the addition of heparin or lyophilised HBP to epididymal spermatozoa and ejaculated spermatozoa has significantly increased the rate of acrosome reaction in both the species.

The present study also showed that rabbits inseminated with epididymal spermatozoa without the addition of HBP produced significantly higher mean littered rates and mean litter born per doe as compared to those animals inseminated with epididymal spermatozoa treated with different levels of HBP. These results showed that epididymal spermatozoa of rabbit could fertilise oocytes without prior exposure to HBP or seminal fluid whose HBP were believed to be necessary for capacitation of spermatozoa in cattle and rabbit.

The group of rabbits inseminated with epididymal spermatozoa treated with HBP at the rate of 4 mg/ml, recorded significantly lower mean litter born per doe as compared to those female rabbits inseminated with epididymal spermatozoa treated with HBP at the lower rates. These results may show that a higher concentration of lyophilized HBP causes more damage to the epididymal spermatozoa instead of further improving their fertilising ability.

Among the four groups of rabbits inseminated with epididymal spermatozoa (ES), epididymal spermatozoa with HBP (ESII), ejaculated spermatozoa (ESM) and ejaculated spermatozoa with HBP (ESMH), there was no significant difference found for the litter rate between the groups inseminated at 0 hour. However, for animals inseminated at 6 hours after induction of ovulation, the litter rate was significantly lower in the group inseminated with ES as compared to the other three groups which were inseminated with spermatozoa which had either natural HBP or added lyophilised HBP. In the present study, it was also found that none of the female rabbits inseminated at 12 and 24 hours after the vaginal stimulation littered.

It was also observed that epididymal spermatozoa samples added with lyophilised HBP bound significantly higher concentration of tritiated (^{3}H) heparin to their membrane as compared to samples that were devoid of HBP. The present study also showed that higher fertile bulls bound significantly higher concentrations of (^{3}H) heparin to their spermatozoa membrane than the lower fertile bulls.

From this study, it can be concluded that (i) epididymal spermatozoa of rabbits could fertilise oocytes without prior exposure to HBP or seminal fluid in the rabbit and (ii) Sahiwal-Friesian bulls could be selected based on their ability to bind (^{3}H) heparin to their spermatozoa.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PENGARUH HEPARIN DAN PROTEIN PELEKAT HEPARIN KE ATAS PESTASI PEMBIAKAN LEMBU JANTAN SAHIWAL-FRIESIAN DAN ARNAB

Oleh

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Suatu kajian pencirian protein pelekat heparin (HBP) dalam semen dari lembu jantan Sahiwal-Friesian dan arnab telah dijalankan. Pengaruh HBP ke atas perstasi pembiakan lembu jantan Sahiwal-Friesian dan arnab juga dikaji.

Dengan menggunakan dimensi satu sodium dodecyl sulfate-polyacrylamide (SDS-PAGE) gel electrophoresis, kelas utama HBP dalam semen telah dikenalpasti dan diasingkan dari lembu jantan Sahiwal-Friesian dan arnab masing-masing mempunyai berat molekular 15, 25, 30, 38 dan 42, 15, 25. 38, 42 dan 101 kDa. Dari kajian ini didapati bahawa lembu jantan yang mempunyai kesuburan yang berbeza mengandungi kelas HBP yang sama dalam semen. Ini mungkin menunjukkan bahawa kehadiran kelas-kelas HBP yang berbeza dalam

semen mungkin tidak boleh digunakan sebagai penunjuk kesuburan sesekor lembu jantan.

Heparin dan HBP yang dibeku kering didapati tidak mempunyai sebarang kesan ke atas peratus motiliti sperma lembu jantan Sahiwal-Friesian dan arnab. Walaubagaimanapun, penambahan heparin atau HBP yang dibeku kering ke atas sperma epididimis dan sperma terejakulat telah memberikan penambahan yang signifikan dalam kadar reaksi akrosom kedua-dua spesis tersebut.

Kajian ini juga menunjukkan bahawa arnab yang disuntik dengan sperma epididimis tanpa penambahan HBP menghasilkan min kadar kelahiran dan min kelahiran anak bagi seekor yang tinggi berbanding dengan ternakan-ternakan yang disuntik dengan sperma epididimis yang telah ditambah dengan kuantiti HBP yang berbeza. Keputusan-keputusan ini menunjukkan bahawa sperma epididimis arnab boleh mempersenyawakan ovum tanpa didedahkan kepada HBP atau plasma semen terlebih dahulu, walaupun dipercayai bahawa HBP yang ada dalam plasma semen perlu untuk kapasitasi sperma lembu dan arnab.

Kumpulan arnab yang disuntik dengan sperma epididimis yang ditambah dengan HBP pada kadar 4 mg/ml. merekodkan min kelahiran anak bagi seekor yang rendah berbanding dengan anrab betina yang disuntik dengan sperma epididimis yang ditambah dengan HBP pada kadar yang rendah. Keputusan-

keputusan ini mungkin menunjukkan bahawa kepekatan HBP yang tinggi membawa lebih keburukan kepada sperma epididimis daripada meningkatan kesuburannya.

Di antara empat kumpulan arnab yang disuntik dengan sperma epididimis (ES), sperma epididimis ditambah dengan HBP (ESH), sperma terejakulat (ESM) dan spermatozoa terejakulat ditambah dengan HBP (ESMH), tiada perbezaan signifikan dalam kadar kelahiran anak antara kumpulan yang disuntik pada 0 jam selepas aruhan ovulasi. Walaubagaimanapun, bagi arnab-arnab yang disuntik pada 6 jam selepas aruhan ovulasi, kadar kelahiran adalah rendah yang signifikan dalam kumpulan yang disuntik dengan ES berbanding dengan ketiga-tiga kumpulan lain yang disuntik dengan sperma samada mempunyai HBP semulajadi atau HBP yang dibeku kering. Dalam kajian ini juga didapati bahawa tiada seekor pun arnab yang disuntik pada 12 dan 24 jam selepas aruhan ovulasia melahirkan anak.

Diperhatikan juga bahawa sampel-sampel sperma epididimis yang ditambah dengan HBP yang dibeku kering melekat lebih banyak heparin bertritium (^3H) pada membrannya berbanding dengan sampel-sample yang tidak ditambah dengan HBP. Kajian ini juga menunjukkan bahawa sperma dari lembu yang lebih subur melekat kepekatan heparin bertritium ^3H yang tinggi pada membran sperma berbanding dengan sperma dari lembu yang kurang subur.

Dirumuskan bahawa (i) sperma epididimis arnab boleh mempersenyawakan ovum tanpa didedahkan kepada HBP atau plasma semen terlebih dahulu pada arnab dan (ii) lembu jantan Sahiwal-Friesian yang lebih subur boleh dipilih berdasarkan keupayaannya melekat heparin bertritium ^{3}H pada membran sperma.

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The thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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RAMAKRISHNAN A/L PATCHAIAPPAN

Date:

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AI	artificial insemination
AV	artificial vagina
BSE	breeding soundness examination
Ca	calcium
DNA	deoxyribonucleic acid
DPM	disintegration per minute
ES	epididymal spermatozoa
ESH	epididymal spermatozoa with heparin binding proteins
ESM	ejaculated spermatozoa
ESMH	ejaculated spermatozoa with heparin binding proteins
g	gram
GAG	glycosaminoglycan
GnRH	gonadotrophin releasing hormone
HBP	heparin binding proteins
^{3}H	tritiated heparin
hr	hour
IBHK	national animal biotechnology institute
IU	international unit
kDa	kilodaltons
kg	kilogram
M	molar concentration
MARDI	Malaysian agricultural research development institute
mCi	millicurie
min	minutes
ml	milliliter
mo	month
N	sample number
NRR	non-return rate
PBS	phosphate buffer saline
pH	logarithm of the reciprocal of the H ion concentration
rpm	rotation per minute
SDS	sodium dodecyl sulfate-polyacrylamide
SD	standard deviation
TALP	tyrode's albumin lactate pyruvate
TDN	total digestible nutrients
ZP	zona pellucida
µl	microliter

CHAPTER I

INTRODUCTION

The population of cattle and water buffalo in Peninsular Malaysia in 1998 was 742,171 and 148,786, respectively (Department of Veterinary Services, 2001). The industry is growing steadily over the years and has earned a place in national economy. The projected population increase for cattle by the year 2025 is estimated to be 993,639. This positive sign of growth in the cattle industry is expected to contribute more towards agriculture and food production.

Sperm capacitation and the acrosome reaction are requisite events in the process of fertilisation (Bedford. 1983). Heparin, one class of glycosaminoglycans (GAG) found in female reproductive tracts, was reported to induce capacitation and acrosome reactions in spermatozoa for bulls and rabbits (Lenz *et al.* 1983a, b). It has been reported that bovine ejaculated spermatozoa required nine hours of incubation with GAGs to undergo acrosomal reaction *in vitro*, whereas epididymal spermatozoa required 22 hours (Handrow *et al.* 1982; Lenz *et al.* 1982; Lee *et al.* 1985). However, a 20 minutes exposure of epididymal spermatozoa to seminal plasma was found to reduce the time required for the acrosome reaction to nine hours as the ejaculated spermatozoa (Lee *et al.* 1985). It was also found that if solubilised zonae pellucidae were used to induce acrosomal reaction in spermatozoa treated with heparin for four hours, ejaculated

spermatozoa responded with acrosome reaction but epididymal spermatozoa did not (Florman and First, 1988a; Florman *et al.* 1989). The exposure of epididymal spermatozoa to seminal plasma *in vitro* enable those spermatozoa to be capacitated by heparin and to respond to zonae pellucidae with an increase in acrosome reaction in a manner similar to ejaculated spermatozoa (Florman and First, 1988). Lee et al. (1985) further showed that seminal plasma increased the number of binding sites for heparin on epididymal spermatozoa. Miller *et al.* (1987) found that bovine seminal plasma which contains several proteins known as heparin binding proteins (HBP), upon ejaculation bound to spermatozoa and were responsible for the heparin bind ability of the spermatozoa. There is scanty information on the effect of purified seminal HBP on the ejaculated and epididymal spermatozoa in Sahiwal-Friesian bull and rabbit. There is also a lack of information on whether purified seminal HBP exert an effect on spermatozoa to alter their fertilising ability in relation to ovulation in rabbit.

Although it has been shown that seminal plasma had increased the number of heparin binding sites on epididymal spermatozoa (Lee et al. 1985), no study has been carried out to determine whether the purified HBP could bring about the same reaction in epididymal spermatozoa. Furthermore, there is lack of information on the amount of heparin bound to the epididymal and ejaculated spermatozoa treated with purified seminal HBP.