J. Trop. Agric. and Fd. Sc. 34(2)(2006): 373–383

Relationship between bull fertility and binding of ^{3}H heparin to spermatozoa in Sahiwal-Friesian bull

(Hubungan antara kesuburan lembu pejantan dengan keupayaan sperma mengikat ^{3}H heparin pada pejantan Sahiwal-Friesian)

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Key words: spermatozoa, heparin binding proteins, ^{3}H heparin, bull fertility

Abstract

A study was carried out to determine the effect of lyophilised heparin binding proteins (HBP) on the heparin binding ability of bovine epididymal spermatozoa and to determine the relationship between bull fertility and total binding of heparin to spermatozoa membrane. It was found that epididymal spermatozoa samples added with lyophilised HBP bound significantly higher (p <0.05) concentration of ^{3}H heparin to their membrane as compared to samples which were devoid of HBP. Spermatozoa from more fertile bulls were found to bind significantly higher concentration of ^{3}H heparin than the less fertile bulls. There was no relationship found between the post-thaw motility of the semen samples and the fertility status of the experimental bulls. Similarly, there was no significant correlation (r = 0.47, p >0.05) between post-thaw motility percentage of semen and heparin binding affinity of spermatozoa from the bulls. This showed that bulls with semen of high motility are not necessarily more fertile than those bulls with lower motility percentage.

Introduction

Sperm capacitation and acrosome reaction are requisite events for the process of fertilisation (Bedford 1983). Heparin, a class of glycosaminoglycans (GAG) found in the female reproductive tract, has been reported to induce capacitation and acrosome reactions in spermatozoa of bulls (Lenz et al. 1983a) and rabbits (Lenz et al. 1983b). It has been reported that bovine ejaculated spermatozoa requires 9 h of incubation with GAGs (heparin) to undergo acrosomal reaction in vitro (Handrow et al. 1982) whereas epididymal spermatozoa requires 22 h (Lee et al. 1985). However, a 20 minute preexposure of epididymal spermatozoa to seminal plasma was found to reduce the time required for acrosome reaction to 9 h, similar to that of the ejaculated spermatozoa (Lee et al. 1985).

When solubilised zonae pellucidae is used to induce acrosomal reaction in spermatozoa treated with heparin for 4 h, ejaculated spermatozoa responds with acrosome reaction but epididymal spermatozoa does not (Florman and First 1988; Florman et al. 1989). The exposure of epididymal spermatozoa to seminal plasma

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in vitro enables those spermatozoa to be capacitated by heparin and responds 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 increases the number of binding sites for heparin on epididymal spermatozoa.

Miller et al. (1987) found that bovine seminal plasma contains several proteins known as heparin binding proteins (HBP), which upon ejaculation, bound to spermatozoa and are responsible for the heparin binding ability of the spermatozoa. The seminal plasma increases the number of heparin binding sites on epididymal spermatozoa (Lee et al. 1985). However, it has not been studied whether the purified HBP could bring about the same reaction in epididymal spermatozoa. There is also scanty information on the effects of purified seminal HBP on ejaculated and epididymal bovine spermatozoa, especially Sahiwal-Friesian bulls.

In cattle breeding, although males are considered half the herd, there is lack of research carried out in Malaysia to determine the most fertile bulls for natural breeding and artificial insemination (AI). Traditional methods of evaluating bull fertility relied on physical measurements and evaluation of semen samples under the light microscope. Although dairy farmers have improved herd fertility to a certain extent by these methods, these measurements usually miss subtle differences in fertility among bulls. Thus, it is imperative to carry out a study to determine whether the heparin binding affinity of the ejaculated spermatozoa could be used as a tool to select bulls of higher fertility. Therefore, the objectives of the study were: a) to determine the effects of purified HBP on the heparin binding ability of epididymal spermatozoa from Sahiwal-Friesian bulls, and b) to determine the relationship between bull fertility and heparin binding ability of the ejaculated spermatozoa.

Materials and methods

Experiment 1

This experiment was carried out to study the effects of lyophilised HBP on the heparin binding ability of bovine epididymal spermatozoa.

Isolation of heparin binding proteins

Newly purchased heparin coupled Sepharose beads (Affi-Gel Heparin Gel, Bio-Rad, Hercules, (CA) was washed three times in phosphate buffered saline (PBS). To wash the beads, one to two bed volumes of cold PBS was added and mixed gently. Then the beads were pelleted by centrifugation at 500 xg and the supernatant was removed using a disposable pipette.

A sample of 2 ml of seminal fluid, which was collected from a vasectomised bull, was added to 10 ml of washed beads in a 50 ml plastic tube. This was incubated for one hour at 4 °C with gentle agitation by using a rocker. After incubation, the beads were centrifuged at 500 x g at 4 °C for 10 min to pellet the beads. The supernatant (unbound protein) was pipetted out. To wash the beads, 30 ml of PBS (4 °C) was added to the beads and mixed gently by inverting the tube for a few times. Then the tubes were centrifuged at 500 x g at 4 °C for 10 min. The supernatant was pipetted out again. This process was repeated three times. To elute the HBP from the heparin coupled Sepharose beads, 5 ml of cold (4 °C) 2 M sodium chloride was added to the washed beads and mixed gently by inverting the tube for a few times. Then the beads were incubated for 30 min at 4 °C with gentle agitation by using a rocker. After incubation, the beads were pelleted by centrifugation at 500 x g at 4 °C and the supernatant (HBP) was removed using a disposable pipette.

Sodium chloride was removed from HBP by dialysis in Spectra/Por molecular porous membrane tubing (Spectrum Medical Industries, Houston, TX) with the molecular weight cut-off of 3,500 in 2 litres of 40 mM ammonium bicarbonate buffer (Sigma

Chemical, St. Louis, MO) for 24 h at 4 °C with two changes of buffer. Dialysed HBP were lyophilised (freeze dried) and stored as powder at –20 °C. Heparin binding proteins were isolated from seminal plasma of five vasectomized and ten unvasectomized Sahiwal-Friesian bulls.

Isolation of epididymal spermatozoa from Sahiwal-Friesian bulls

Cauda epididymides were removed surgically from four apparently healthy Sahiwal-Friesian bulls aged between three and four years old. The epididymectomy was performed successfully under a sedative [2-(2.6-xylidino)-5, 6 dihydro-4H-1, 3-thiazine-hydrochloride, Rompun solution 2%, Bayer Leverkusen] of 2 ml injected intramuscularly, 15 min before the operation. The animal was fast and abstained from water for at least 12 h before sedation.

The operation to collect epididymal spermatozoa was performed on the standing position of the animal. The scrotum was cleaned and swabbed with tincture of iodine. The incision of skin of about 2.5 cm was made along the ventral aspect of the scrotum over the tail of the epididymis while forcing the testicle of the bottom of the scrotum using the other hand of the surgeon. The incision was deepened until the testicle was exposed. The testicle was pulled out of the scrotum and the cauda epididymis was located. The greyish-white cauda epididymis was about 1.5 x 3.0 cm long. The vas deferens was clamped with an artery forceps and was ligated above and below the forceps with chromic catgut. Similar clamp and ligature were also carried out at the attachment of the cauda epididymis with the testicle. The cauda epididymis was then removed by cutting between the ligatures and immediately immersed into 40 ml of Tris solution [3.02% hydroxy methyl-amino methane, 1.68% citric acid, 1.25% D (–) fructose, 1,000 IU/ml penicillin, 1.0 mg/ml streptomycin] in a water bath at 37 °C. The testicle was pushed back into the scrotum and the incision site was closed with interrupted sutures. The scrotal skin was sprayed with an antiseptic and a fly repellent.

The freshly isolated cauda epididymal tissue was washed in Tris buffer and then minced in egg yolk Tris extender using a pair of sharp scissors. The minced tissue was kept in a water bath at 37 °C for 15 min. The supernatant was then pipetted out and analysed for the progressive motility and sperm concentration as described by Ramakrishnan et al. (1989). More extender (37 °C) was added to the sperm samples in order to make the concentration of the sperm to 100 x 10^6 per ml. Only sperm samples with more than 35% progressive motility were used for this study.

Semen extender

The Tris buffered egg yolk extender was prepared by combining 20% egg yolk to 80% of Tris buffer by volume.

The composition of Tris buffer used in this study is as follows:

Tris (Hydroxy methyl-aminomethane)	30.20 g
Citric acid	16.75 g
D (–) Fructose	12.50 g
Penicillin	1,000 IU/ml
Streptomycin	1.00 mg/ml
Distilled water added to	1,000 ml

Semen evaluation

Progressive motility and spermatozoa concentration were carried out using an Olympus BH2 phase contrast microscope. Progressive motility of epididymal spermatozoa was determined by applying a coverslip on a drop of extended epididymal spermatozoa on a warm slide. The slide was examined under high power (400x).

A haemocytometer was used to determine spermatozoa concentration. A sample of 0.1 ml of semen was added into 9.9 ml of formal saline in a test tube. The content was mixed thoroughly. A coverslip was placed on the haemocytometer counting chamber followed with a drop of the diluted semen. About 5–10 min after preparing the

haemocytometer, the total number of spermatozoa was counted in five large squares and multiplied by 5 x 10^6 to obtain the spermatozoa concentration per ml.

Heparin binding assay

To study the effects of HBP on epididymal spermatozoa, the evaluated and extended epididymal spermatozoa sample was devided into two equal parts and one of the parts was added with lyophilized bull HBP (3 mg/ml) and mixed gently using a glass rod. All the samples were then incubated at 37 °C for 30 min as recommended by McCauley et al. (1996). After 30 min, aliquots of the epididymal spermatozoa samples were taken and evaluated for progressive motility.

Each sample of 2 ml was pipetted into a test tube. In each sample, 2 ml TALP medium [NaCl 137 mM, $CaC1_2$ 1.8 mM, KCl 2.6 mM, $MgCl.6H_2O$ 0.23 mM, NaH_2PO4 0.36 mM, NaHCO3 0.25 µM, HEPES 20 mM, lactic acid 21.6 mM, pyruvate 45 µM, 0.6% BSA (W/V; Fraction V, Scientific Protein Laboratories, Waunakee, WI), 100 units/ml of penicillin, and 100 µg/ml of streptomycin, pH 7.4] were added and then incubated at room temperature for 20 min. After incubation, to wash the spermatozoa, the tubes were centrifuged at 1,200 rpm (800 xg) for 10 min at 4 °C. The supernatant was pipetted out and 2 ml of TALP medium at 4 °C was added to the samples. The samples were then vortexed and centrifuged as before. After washing the epididymal spermatozoa samples for a total of three times, each sample was then diluted with TALP to a concentration of 2 x 10^6 cells/ml.

Multiscreen 0.22 µm 96-well filtration plates (Millipore, Molsheim, France) were placed on a vacuum manifold and washed three times by adding 100 µl of TALP into the wells and vacuumed out. Diluted epididymal spermatozoa sample of 100 µl with the spermatozoa concentration of 2 x 10^6 cells/ml (200,000 cells) were placed into 12 x 2 wells. Then the wells were vacuum filtered. The cells in the wells were then washed over the filter by vacuum filtration 3 times with 100 µl of TALP.

Saturation binding assays were performed under equilibrium conditions (Handrow et al. 1982). A total of 12 serial dilutions of tritiated heparin (^{3}H heparin) (specific activity 0.41 mCi/mg, New England Nuclear, Boston, M.A.) ranging from 0.79 to 1,626 pmol in 100 µl of Tris buffer were added to all the spermatozoa samples.

To carry out the non-specific binding assay, one of the rows of 12 wells was then added with 100 µl of TALP. The wells in the other rows were added with 100 µl of 12 serial dilutions of sodium heparin salt (Sigma Chem Co.). The concentration of the sodium heparin salt solution was 100 times as that of the heparin in the ^{3}H heparin solution in every well.

By adding 100 µl of TALP to one row of the wells and 100 µl of 12 serial dilutions of sodium heparin salt to the wells in another row, all wells were brought to a final volume of 200 µl. The plate was covered and then wrapped with aluminium foil and kept at 4 °C for about 12 h. After the incubation, the plate was unwrapped and filtered by vacuum filtration using the vacuum manifold. Then the spermatozoa on the filters of all the wells were washed three times with 100 µl of TALP at 4 °C. The plate was then taken out of the vacuum manifold and placed on a tissue paper so that the bottom of the plate was wet-free. The filters were then punched from the plates into scintillation vials with 2.5 ml of Readysafe Scintillation Cocktail (Beckman). After incubating the vials for about 3 h at room temperature, the vials were counted in a Beckman LS6000SC Liquid Scintillation Counter. The disintegration per minute (DPM) counts was automatically calculated using a program that comes with the counter. Based on the activity of the ^{3}H heparin, results were corrected to heparin bound to the spermatozoa in pico moles (pmol). The treatment was replicated two

times for each epididymal spermatozoa sample. The saturation binding assays on both the split spermatozoa samples were carried out simultaneously.

The non-specific binding assays were performed with the assumption that the labelled and unlabelled heparin compete equally for the binding sites on the sperm membrane (Marks and Ax 1985). The heparin bound in the non-specific binding was subtracted from the total heparin bound in the specific binding to get the actual (total) binding of the ^{3}H heparin to epididymal spermatozoa of the bulls. This can be rewritten as follows:

Total heparin bound
= Specific binding (pmol) – Non-specific binding (pmol) to spermatozoa (pmol)

Experiment 2

This eperiment was carried out to determine the relationship between bull fertility and total binding of heparin to their spermatozoa membrane.

Semen samples and breeding data of Sahiwal-Friesian bull were collected from the National Animal Biotechnology Institute (IBHK), Bukit Dinding, Jerantut, Malaysia. Egg yolk Tris extender was used as the extender to freeze semen samples of the bulls. The frozen samples were sent through out Malaysia for inseminating cows that belong to smallholders, commercial as well as government farms.

Rancangan Tenusu, Padang Hijau, Kluang, Johor is one of the government farms that uses frozen semen from IBHK for artificial insemination. In this farm, the frozen semen is being used at random on cows of various ages. Based on the fertility performance of the frozen semen in this farm, breeding data of nine Sahiwal-Friesian bulls aged between 4 and 5 years old were collected.

A total of 25 frozen semen straws belonging to each of the nine bulls were transported in liquid nitrogen from IBHK to MARDI station and stored in deep freeze (–196 °C) pending analysis.

In the laboratory, five straws from each bull were thawed in a water bath at 37 °C for 30 s. Then the contents were combined and incubated at 37 °C. Aliquots were taken and evaluated for semen post-thaw motility. Each sample was then added to 2 ml of TALP medium and was incubated at room temperature for 20 min.

After incubation, to wash the spermatozoa, the tubes were centrifuged at 1,200 rpm (800 xg) for 10 min at 4 °C. The supernatant was pipetted out and 2 ml of TALP medium (4 °C) was added to the samples. The samples were then vortexed and centrifuged as before. After washing the semen samples for a total of 3 times, each sample was then diluted with TALP to a concentration of 2,000,000 cells per ml.

All saturation binding assays and nonspecific bindings were performed in duplicate using multiscreen 0.22 µm 96-well filtration plates and twelve serial dilutions of tritiated heparin (^{3}H heparin) as described in Experiment 1.

The experiment was replicated 3 times for the semen samples from each animal. The heparin bound in the non-specific binding was subtracted from the total heparin bound in the specific binding to get the actual binding of the ^{3}H heparin to spermatozoa of the bulls as calculated above.

Grouping of hulls according to their fertility status

The nine experimental bulls were divided into three groups according to their fertility status, namely, low, average and high. The fertility status of the bulls was determined based on the number of females inseminated with their frozen semen and the number of animals calved *(Table 1)*.

Table 1. Fertility status of nine Sahiwal-Friesian bulls

Bulls	Semen post-thaw motility (%)	Number of services	Female calved (%)	Fertility status of bulls
A	30	200	23.0	Low
B	35	466	24.5	Low
C	35	121	24.8	Low
D	40	152	27.6	Average
E	25	764	28.4	Average
F	35	383	31.6	Average
I	35	109	33.0	High
H	35	589	37.7	High
G	40	84	41.7	High

Table 2. Mean concentration of tritiated heparin (^{3}H) bound to epididymal spermatozoa (treated with and without heparin binding proteins) of Sahiwal-Friesian bulls (mean* of 4 bulls) in a 12 serial dilutions of tritiated heparin

Concentration of ^{3}H (pmol in 100 µl)	^{3}H bound to epididymal spermatozoa (pmol)			
	N	Without HBP	N	With HBP
1,626.00	8	2.57 ± 0.54a	8	2.90 ± 0.54b
813.00	8	2.20 ± 0.69a	8	2.45 ± 0.54b
405.50	8	1.13 ± 0.26a	8	1.77 ± 0.78b
203.30	8	1.07 ± 0.30a	8	1.61 ± 0.65b
101.60	8	0.77 ± 0.29a	8	1.18 ± 0.56b
50.81	8	0.65 ± 0.29a	8	1.00 ± 0.56b
25.41	8	0.51 ± 0.24a	8	0.79 ± 0.44b
12.70	8	0.38 ± 0.14a	8	0.60 ± 0.27b
6.35	8	0.31 ± 0.12a	8	0.53 ± 0.32b
3.18	8	0.26 ± 0.13a	8	0.40 ± 0.22a
1.59	8	0.18 ± 0.09a	8	0.32 ± 0.19a
0.79	8	0.15 ± 0.06a	8	0.24 ± 0.15a

HBP = Heparin binding proteins
*Two semen samples of each bull were analysed
Means in the same row with different letters are significantly different (p <0.05)

Percentage of female calved was calculated as follows:

$$\text{No. of cows calved} = \frac{\text{Percentage of cows calved}}{\text{No. of cows inseminated with frozen semen} \times 100}$$

Analysis of data

The data collected was analysed statistically (Steel and Torrie 1960; SAS Inst. 1982). Analysis of variance was done using a general linear model. The difference between means was tested using Duncan Multiple Range Test.

Results and discussion

Mean concentration of ^{3}H heparin bound to epididymal spermatozoa of Sahiwal-Friesian bulls in 12 serial dilution of ^{3}H heparin is shown in *Table 2*. Epididymal spermatozoa samples with HBP bound significantly higher (p <0.05) concentration of ^{3}H heparin to their membrane as compared to samples which were devoid of HBP. These results showed that HBP might regulate the epididymal spermatozoa to bind more ^{3}H heparin on their membrane. Nass et al. (1990) found that seminal plasma alters the ability of epididymal spermatozoa to bind heparin. These workers further suggested

that the several HBP found in bull seminal plasma could be the actual factors that were responsible for increasing the ability of epididymal spermatozoa to bind heparin. Miller et al. (1990) reported that bovine spermatozoa that have been exposed to seminal plasma possess more binding sites for heparin than spermatozoa from the cauda epididymis that have not been exposed to accessory sex gland secretions. Seminal plasma exposure enables spermatozoa, following incubation with heparin, to undergo acrosome reaction. The present study proved that the ability of epididymal spermatozoa to bind heparin could be increased by adding purified seminal HBP instead of seminal plasma.

In the present study, it was also found that the epididymal spermatozoa, which were devoid of HBP, continued to bind more ^{3}H heparin with the increment of ^{3}H heparin added in the 12 serial dilutions although the concentration bound was significantly lower as compared to the epididymal spermatozoa exposed to HBP *(Table 2)*. These results further showed that bovine epididymal spermatozoa have the ability to bind heparin to their membrane even without the help from HBP. However, the present results showed that HBP could increase the ability of the epididymal spermatozoa to bind significantly more heparin to their membrane. Since, heparin stimulates capacitation, and HBP play a major role in increasing the ability of the spermatozoa in binding more heparin to their membrane, this may show that HBP increase the ability of the spermatozoa in binding more heparin-like glycosaminoglycans in the female reproductive tract to induce greater percentage in capacitation and subsequently increase the fertilisation rate. The present results were similar to the findings of Florman and First (1988). These workers reported that the exposure of epididymal spermatozoa to seminal plasma in vitro enables those spermatozoa to be capacitated by heparin and to respond to zonae pellucidae with an increase in acrosome reactions in a manner similar to ejaculated spermatozoa.

It has been reported that ejaculated sperm required 9 h of incubation with GAGs to undergo acrosomal reaction without addition of zonae pellucidae, whereas epididymal spermatozoa with GAGs required 22 h (Handrow et al. 1982; Lee et al. 1985). These findings indicate that epididymal spermatozoa with GAGs can undergo acrosomal reaction, which is necessary for fertilisation even without exposing the epididymal spermatozoa to seminal plasma or HBP. In a previous study, it has been shown that in rabbit, epididymal spermatozoa not only can fertilise ovum in vivo without prior exposing them to seminal plasma or HBP, the litter rate and litter born per doe were comparable to those of

Table 3. Overall mean* concentration of tritiated heparin (^{3}H) bound to epididymal spermatozoa (treated with and without heparin binding proteins) of four Sahiwal-Friesian bulls in 12 serial dilutions of tritiated heparin

Bull	Epididymal spermatozoa without HBP			Epididymal spermatozoa with HBP		
	N	^{3}H bound (pmol)	Sperm motility (%)	N	^{3}H bound (pmol)	Sperm motility (%)
A	24	0.55 ± 0.53Aa	40	24	0.71 ± 0.67Ba	40
B	24	1.0 ± 0.93Ab	35	24	1.11 ± 0.93Bb	35
C	24	1.11 ± 0.91Ab	40	24	1.71 ± 0.98Bb	40
D	24	0.76 ± 0.68Ac	40	24	1.06 ± 0.89Bc	40

HBP = Heparin binding proteins

*Mean of the 12 serial dilutions of tritiated heparin added

Means in the same row with different capital letters are significantly different (p <0.05)

Means in the same column with different small letters are significantly different (p <0.05)

ejaculated spermatozoa (Ramakrishnan et al. 2000).

Table 3 shows the overall mean concentration (mean of the 12 dilutions) of ^{3}H heparin bound to epididymal spermatozoa (with and without the addition of HBP) of four Sahiwal-Friesian bulls in 12 serial dilutions of ^{3}H heparin. In addition, percentages of motile spermatozoa in the samples of each bull are also shown in *Table 3.*

Binding assay results from the four bulls showed that there was significant difference ($p < 0.05$) among the four bulls in the binding ability of ^{3}H heparin. In bull A, the mean concentration of ^{3}H heparin bound by the epididymal spermatozoa without the treatment of HBP was only 0.55 ± 0.53 pmol. However, epididymal spermatozoa devoid of HBP from bull C could bind as high as 1.11 ± 0.91 pmol of ^{3}H heparin. In bulls B and C, the amount of ^{3}H heparin bound to epididymal spermatozoa with and without HBP were significantly higher ($p < 0.05$) as compared to bulls A and D. This study showed that the epididymal spermatozoa which could bind a higher concentration of ^{3}H heparin without the addition of HBP, also could bind proportionally higher concentration of ^{3}H heparin with the addition of HBP. All the samples with the addition of HBP bound significantly higher ($p < 0.05$) amount of ^{3}H heparin as compared to samples which were without HBP.

It was also found that there was no relationship between the percentages of motile spermatozoa and the ^{3}H heparin binding ability of the samples. Although the sample from bull B contained only 35% of motile spermatozoa, it bound significantly higher ($p < 0.05$) amount of ^{3}H heparin as compared to bulls A and D whose samples contained 40% motile epididymal spermatozoa. Similar results were also reported by McCauley et al. (1996) in *Bos taurus* beef bulls. The present study also showed that there was a variation in the ^{3}H heparin binding ability of epididymal spermatozoa from different bulls. Similar results were also reported by Ax and Lenz (1987).

Table 4 shows the binding of ^{3}H heparin to ejaculated spermatozoa of nine Sahiwal-Friesian bulls to a concentration of 1,626 pmol of ^{3}H added, the number of services of each bull and the percentages of animals calved. Spermatozoa from the higher fertile bulls usually bound significantly higher ($p < 0.05$) concentration of ^{3}H heparin than the lower fertile bulls. Spermatozoa from bulls A and B, whose fertility were the lowest among the nine bulls, bound significantly lower ($p < 0.05$) concentration of ^{3}H heparin as compared to the other seven bulls. These findings may indicate that spermatozoa from high fertile bulls posses the ability to bind more heparin or heparin-like materials from the female reproductive tract and capacitate faster thus increased the chances for the spermatozoa to fertilise the ovum. This phenomenon could be the factor that makes the bull more fertile. Ax and Lenz (1987) also reported that of high fertility bulls produce spermatozoa that undergo significantly greater percentages of acrosome reaction in response to heparin-like materials than spermatozoa from lower fertility bulls.

In the present study, it was found that there was no relationship between motility of post-thawed semen motility and the fertility status of the bulls *(Table 4)*. It was also found that there was no significant correlation ($r = 0.47$, $p > 0.05$) between post-thaw motility percentage of semen and heparin binding affinity of spermatozoa from the nine bulls. This showed that it is not necessary that bulls with semen of high motility percentage are more fertile than those bulls with lower motility percentage.

The ability of spermatozoa to bind ^{3}H heparin in the low fertile bulls was significantly lower ($p < 0.05$) compared to the average and high fertile bulls *(Table 5)*. There was no significant difference ($p > 0.05$) between the mean post-thawed motility of the semen samples from the three

Table 4. Binding of ^{3}H heparin to spermatozoa of nine Sahiwal-Friesian bulls to a concentration of 1,626 pmol of ^{3}H added

Bull*	Semen post-thaw motility (%)	Number of services	Female calved (%)	^{3}Heparin bound to spermatozoa (pmol)
A	30	200	23.0	1.99 ± 0.10f
B	35	466	24.5	2.52 ± 0.12ef
C	35	121	24.8	4.69 ± 0.33bc
D	40	152	27.6	5.56 ± 0.32a
E	25	764	28.4	4.18 ± 0.52d
F	35	383	31.6	4.08 ± 0.37ab
I	35	109	33.0	3.04 ± 0.16e
H	35	589	37.7	4.64 ± 0.53cd
G	40	84	41.7	4.91 ± 0.26bc

Bulls A, B and C were designated as low fertility; Bulls D, E and F were designated as average fertility; bulls G, H and I were designated as high fertility
Means in the same column with different letters are significantly different (p <0.05)

Table 5. Tritiated heparin binding abilities of ejaculated spermatozoa* from bulls of low, average and high fertility using a ^{3}H heparin concentration of 1,626 pmol

Fertility status of bulls	N	Semen post-thaw motility (%)	Number of services	Female conceived (%)	^{3}Heparin bound to spermatozoa (pmol)
Low	3	33.33 ± 2.36	787	24.1 ± 3.0a	3.13 ± 1.27a
Average	3	31.67 ± 6.23	1,299	29.2 ± 1.73b	4.56 ± 0.58ab
High	3	36.67 ± 2.36	782	37.47 ± 3.56c	4.20 ± 0.90b

*Mean of 3 bulls of similar fertility status
Means in the same column with different letters are significantly different (p <0.05)

groups of bulls used in this study. However, the pregnancy rates of the three groups were significantly different (p <0.05).

In contrast to the present results, Ax and Gilbert (1987) reported that the binding affinity of heparin decrease significantly as irregularites in ejaculated semen samples increase. However, these workers used raw semen for their work while in the present study, frozen semen samples were used. It is an established fact that more than 45% of the live spermatozoa would die during the freezing process (Ramakrishnan and Adnan 1992). So during freezing process, all the irregular or abnormal spermatozoa must have died leaving only the normal spermatozoa to survive the process. This could be the reason of no relationship between the post-thaw motility of the semen samples and the binding affinity of ^{3}H heparin to bull spermatozoa in the present study.

Conclusion

The ability of the epididymal spermatozoa to bind heparin could be increased by adding purified seminal HBP. There was no relationship between post-thawed semen motility and the fertility status of the bulls, but spermatozoa of higher fertility bulls were found to bind higher concentration of ^{3}H heparin as compared to the lower fertility bulls. Therefore, in selection of higher fertility bulls, more emphasis should be given to the ability of the spermatozoa of the bull to bind more heparin rather than selecting a bull based on its semen motility. However, further study has to be carried out with a larger number of breeding bulls to confirm these findings.

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Abstrak

Satu kajian telah dijalankan untuk menentukan kesan protein pengikat heparin (HBP) yang dibeku kering terhadap keupayaan sperma epididimis lembu membentuk ikatan dengan heparin. Kajian ini juga dijalankan untuk menentukan hubungan antara kesuburan lembu pejantan dengan jumlah ikatan heparin pada membran spermanya. Didapati bahawa sperma epididimis yang ditambah dengan HBP yang dibeku kering mengikat lebih banyak heparin bertritium (^{3}H) pada membrannya berbanding dengan sampel yang tidak ditambah dengan HBP. Tiada hubungan antara motiliti sampel semen selepas dinyahsejuk beku dengan status kesuburan lembu pejantan yang dikaji. Hubungan antara peratus motiliti semen selepas dinyahsejuk beku dengan keupayaan mengikat heparin didapati tidak signifikan ($r = 0.47$, $p > 0.05$). Ini menunjukkan bahawa lembu pejantan yang mempunyai motiliti semen yang tinggi tidak semestinya lebih subur daripada pejantan yang mempunyai peratus motiliti yang rendah.

Accepted for publication on 18 November 2005