
**UNIVERSITI PUTRA MALAYSIA**

# *ASSESSMENT OF BALI CATTLE SEMEN USING DIFFERENT COLLECTION METHODS, ULTRASTRUCTURAL CHANGES OF SPERMATOZOA, AND RELATIONSHIP BETWEEN SEMINAL PLASMA PROTEINS AND IN VITRO FERTILITY*

**KAJAL SARSAIFI**

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# ASSESSMENT OF BALI CATTLE SEMEN USING DIFFERENT COLLECTION METHODS, ULTRASTRUCTURAL CHANGES OF SPERMATOZOA, AND RELATIONSHIP BETWEEN SEMINAL PLASMA PROTEINS AND IN VITRO FERTILITY

**By**

**KAJAL SARSAIFI**



**Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfillment of the Requirements for the Degree of Doctor of Philosophy**

**February 2013**

## DEDICATION

I dedicate this thesis to my mother Fereshteh
Who has raised me to be the person who I am today. Thank you for all the unconditional love, guidance and support that you have always given me

To my two dearest brothers Semko and Danial and to my lovely sister Delaram, who have given lots of love and support to the continuance my education

To my beloved husband,
Homayoun Hani, for his immense support, patience, and encouragement.

and I dedicate this thesis
To my late father, God bless his soul

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement of the degree of Doctor of Philosophy

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**February 2013**

**Chairman:** **Prof. Abd. Wahid Bin Haron, D.V.M., PhD**

**Faculty:** **Veterinary Medicine**

Male infertility is a major issue in mammalian reproduction and thus, it is important to ascertain that a bull is fertile prior to breeding. The potential fertility of a bull can be determined by field fertility rates. However, this procedure of determining fertility is expensive and time-consuming. Therefore, the present study was designed to develop a method for the prediction of frozen-thawed Bali cattle spermatozoa fertility using various sperm characteristics and heterologous *in vitro* fertilization (IVF) of zona-free hamster oocytes (ZFHOs). The main objective of the study was to evaluate the protein composition of the seminal plasma of Bali bulls and the proteins related to bull fertility. Semen samples were collected from 25 untrained Bali bulls using three different collection methods namely electrical ejaculation (EE), rectal massage (RM) and a combination of RM and EE. The effects of these methods on pre- and post-

thawed semen characteristics were evaluated. All specimens were assessed for motility characteristics, capacitation, acrosome and membrane integrities, acrosome reaction, and ultrastructure. To assess the fertility of frozen-thawed bull spermatozoa, heterologous IVF using ZFHOs was conducted. To improve sperm penetration into ZFHOs, three sperm separation methods namely Swim-up, Percoll® and BoviPure®, were used. Prior to co-incubation with ZFHOs, the spermatozoa were first incubated in four media containing 0, 25, 50 and 100 µg/ml heparin. The relationship between different motions of sperm characteristics semen assessed by CASA and the percentage of IVF success were also studied. In another study, the seminal plasma proteins (SPP) were separated using 2-dimensional SDS-PAGE followed by Coomassie blue staining and the polypeptide maps were analyzed using the Image Master Software. Proteins were identified by Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS/MS). The results of the study showed that EE and combination method (RM-EE) can be used to obtain semen from Bali cattle, with a 100% success rate with the EE method. Post-thawed semen collected using the EE method was also of acceptable quality that would increase the chance of conception, and subsequently can be used in the conservation of Bali cattle. Better fertilization percentages (FP) and fertilization indexes (FI) were observed in ZFHOs inseminated with spermatozoa separated by Swim-up and BoviPure® compared to the Percoll® method. In addition the higher heparin concentrations (50 and 100 µg/ml) with 30 min incubation time improved heterologous IVF rates (FP: 68.42-74.64 % and FI: 3.15-3.73). With regards to FP and FI, significant differences ($P<0.05$) were observed between bulls from heterologous IVFs of ZFHOs with Swim-up spermatozoa and

BoviPure® spermatozoa group. Among the spermatological and IVF parameters, FP ($r^2$= 0.930) and FI ($r^2$= 0.875) were identified as being the most predictive of bull fertility and have high correlations with acrosome reaction ($r^2$=0.830). The results suggest that heterogeneous IVF using ZFHOs is potentially an informative method for assessing *in vivo* fertilization ability of Bali bulls. Bulls of high fertility had significantly higher mean values of sperm characteristics compared to infertile bulls. Statistical analysis of percentage motility, morphology and FP showed that the correlation coefficient between sperm qualitative characteristics and overall bull fertility were significant ($P<0.05$). Based on these findings, the proteomics profiling of seminal plasma of Bali bulls have significant merit. An average of 116 ± 8 spots was detected on the SPP gels. An interesting finding in this study is that there were three spots, namely 9, 13 and 16 (MW: 14 to 16 kDa, and PI: 4.9 to 5.8), which were generally more significantly intense in the infertile group than in the fertile group. Upon MALDI-TOF-MS/MS analysis, these three spots were identified as a type of bovine SPP (PBSA1/A2) known as PDC-109 that was up-regulated ($P<0.05$) in the infertile Bali cattle group. The PDC-109 is involved in sperm capacitation and fertilization and its abundance is associated with fertility of Bali bulls. Spots 3 and 5, identified as sperm-adhesion proteins (MW: 13 to 15 kDa and PI: 5 to 5.8) were not significant ($P>0.05$) up-regulated in the infertile group. Spots 18 and 68, known as seminal ribonuclease (MW; 14 to 16 kDa and PI; 8.4 to 9.3), were significantly down-regulated ($P<0.05$) in the infertile group. However, spot 68 was found at the edge of the acidic site, so it may not serve as a good potential marker due to the limitation of pI range (3 to 10) in the ReadyStrip™. Spot 79, which is serum albumin, was also down-regulated in the

infertile group. This is the first report on the PDC-109 abundance related to bull fertility. This study is also a first comprehensive description of Bali cattle semen and seminal plasma proteome. These findings suggest that there are several proteins in the Bali cattle seminal plasma fluid which could be related to the fertility of this species.

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

**PENILAIAN SEMEN LEMBU BALI MENGGUNA KAEDAH PENGUMPULAN BERBEZA, PERUBAHAN ULTRASTRUKTUR SPERMATOZOA DAN PERKAITAN DI ANTARA PROTEIN PLASMA SEMEN DAN KESUBURAN *IN VITRO***

Oleh

**KAJAL SARSAIFI**

**Februari 2013**

**Pengerusi : Prof. Abd. Wahid Bin Haron, D.V.M., PhD**

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Kesuburan jantan adalah suatu isu utama dalam pembiakan mamalia dan justeru itu adalah penting untuk memastikan lembu jantan itu subur sebelum melakukan pembiakbakaan. Kesuburan potensi seekor lembu jantan boleh ditentukan daripada kadar kesuburan di padang. Bagaimanapun, prosedur untuk menentukan kesuburan ini adalah mahal dan memakan masa. Dengan demikian, kajian ini direka bentuk untuk mengembangkan suatu kaedah untuk meramalkan kesuburan spermatozoa sejuk beku-nyahsejuk lembu Bali beku mengguna pelbagai ciri sperma dan pensenyawaan *in vitro* (IVF) heterologous untuk oosit hamster bebas-zona (ZFHOs). Objektif utama kajian ini ialah menilai komposisi protein dalam plasma semen lembu jantan Bali dan protein berkaitan kesuburan lembu jantan. Sampel semen dikumpul daripada 25 ekor lembu jantan Bali yang tidak terlatih, mengguna tiga kaedah pengumpulan iaitu pemancutan elektrik

(EE), urut rektum (RM) dan gabungan EE-RM. Kesan kaedah ini terhadap ciri semen pra- dan pasca-nyahsejuk beku dinilai. Semua spesimen dinilai untuk ciri kemotilan, pengkapasita, integriti akrosom dan membran, tindak balas akrosom dan ultrastruktur. Untuk menilai kesuburan spermatozoa lembu jantan nyahsejuk beku, IVF heterologus mengguna ZFHOs dilakukan. Untuk meningkatkan penembusan sperma masuk ZFHOs, tiga kaedah pengasingan sperma iaitu renang-naik, Percoll® dan BoviPure® digunakan. Sebelum dieramkan bersama ZFHOs, spermatozoa terlebih dahulu dieramkan dalam empat media mengandungi 0, 25, 50 dan 100 µg/ml heparin. Perkaitan di antara pelbagi pergerakan ciri sperma dinilai dengan CASA dan peratusan kejayaan IVF juga dikaji. Dalam suatu kajian lain, protein plasma semen (SPP) dipencil mengguna SDS-PAGE 2-dimensi, diikuti dengan pewarnaan Coomassie biru dan peta polipeptida dianalisis mengguna perisian Image Master. Protein dikenal pasti melalui spektrometri jisim masa-terbang nyahjerapan/pengionan terbantu-matriks (MALDI-TOF-MS/MS). Hasil kajian menunjukkan yang kaedah EE dan gabung kaedah RM-EE boleh diguna untuk memperolehi semen daripada lembu Bali, dengan kadar kejayaan 100% melalui kaedah EE. Kualiti semen pascanyahsejuk beku dikumpul mengguna kaedah EE boleh diterima dan akan meningkatkan kemungkinan konsepsi, dan seterusnya boleh diguna dalam pemuliharaan lembu Bali. Peratusan pensenyawaan (FP) dan indeks pensenyawaan (FI) yang lebih baik dilihat pada ZFHOs dipermani dengan spermatozoa yang diasing melalui kaedah renang-naik dan BoviPure® daripada kaedah Percoll®. Disamping itu, kepekatan heparin lebih tinggi (50 dan 100 µg/ml) dengan tempoh pengeraman 30 minit meningkatkan kadar IVF heterologus (FP: 68.42-74.64% dan FI: 3.15-3.73). Merujuk kepada FP dan FI,

perbezaan tererti ($P<0.05$) dilihat di antara lembu jantan daripada kumpulan IVF heterologus ZFHOs dengan spermatozoa renang-naik dan kumpulan spermatozoa BoviPure®. Di kalangan parameter spermatologi dan IVF, FP ($r^2=0.930$) dan FI ($r^2=0.875$) dikenal pasti sebagai yang paling baik dalam meramal kesuburan lembu jantan dan ianya mempunyai korelasi tinggi dengan tindak balas akrosom ($r^2=0.830$). Hasil ini menyarankan yang IVF heterologus mengguna ZFHOs merupakan kaedah yang berpotensi memberi matlumat banyak dalam penilaian keupayaan pensenyawaan *in vivo* lembu jantan Bali. Lembu jantan tinggi kesuburannya mempunyai nilai min ciri sperma lebih tinggi tererti ($p<0.05$) berbanding lembu tak subur. Analisis statistik terhadap peratusan kemotilan, morfologi dan FP menunjukkan bahawa pekali korelasi di antara ciri kualiti sperma dengan kesuburan keseluruhan lembu jantan adalah tererti ($P<0.05$). Berdasarkan penemuan ini, pemprofilan proteomik plasma semen lembu jantan Bali ada merit yang nyata. Secara purata 116 ± 8 bintik dapat dikesan pada gel SPP. Penemuan yang menarik dalam kajian ini ialah wujudnya tiga bintik iaitu 9, 13 dan 16 (berat molekul: 14 hingga 16 dan PI: 4.9 hingga 5.8) yang umumnya lebih terang tererti dalam kumpulan tidak subur daripada kumpulan subur. Apabila analisis MALDI-TOF-MS/MS dilakukan, tiga bintik tersebut dikenal pasti sebagai sejenis SPP bovin (PBS A1/A2) dikenali sebagai PDC-109, yang ternaik kawal ($P<0.05$) dalam kumpulan lembu Bali tidak subur. PDC-109 terlibat dalam pengkapasitatan dan pensenyawaan sperm dan wujudnya dengan banyak adalah berkaitan dengan kesuburan lembu jantan Bali. Bintik 3 dan 5 yang dikenal pasti sebagai protein lekatan sperma (berat molekul: 13 hingga15 dan PI: 5 hingga 6.8) ternaik kawal secara tidak tererti ($P>0.05$) dalam kumpulan tidak subur. Bintik 18 dan 68 yang dikenali

sebagai ribonuklease semen (berat molekul: 14 hingga 16 kDa dan PI: 8.4 hingga 9.3) yang terturun kawal secara tererti ($p<0.05$) dalam kumpulan tidak subur. Bagaimanapun, bintik 68 terdapat pada pinggir tapak berasid, justeru itu ia tidak boleh diguna sebagai penanda berpotensi baik kerana adanya batasan julat pI (3 hingga 10) pada ReadyStrip™. Bintik 79 yang merupakan albumin serum juga terturun kawal dalam kumpulan tidak subur. Ini adalah laporan pertama mengenai banyaknya PDC-109 yang berkaitan kesuburan lembu jantan. Kajian ini juga merupakan huraian komprehensif pertama terhadap semen dan proteom plasma semen lembu Bali. Penemuan ini menyarankan bahawa ada beberapa protein dalam bendalir plasma semen lembu Bali yang mungkin boleh dikaitkan dengan kesuburan spesies ini.

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I certify that a Thesis Examination Committee has met on 27th of Jun 2013 to conduct the final examination of Kajal Sarsaifi on her thesis entitled "Assessment Of Bali Cattle Semen Using Different Collection Methods, Ultrastructural Changes Of Spermatozoa, And Relationship Between Seminal Plasma Proteins And In Vitro Fertility" in accordance with the universities and university colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of Supervisory committee are as follows:

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## DECLARATION

I declare that the thesis is on my original work except for quotation and citation, which have been duly acknowledged. I also declare that it has not been previously, and it is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institutions.

**KAJAL SARSAIFI**

Date: 27 Jun 2013

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