

LIVESTOCK BREEDING
THE PAST,
THE PRESENT
AND THE FUTURE



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LIVESTOCK BREEDING THE PAST, THE PRESENT AND THE FUTURE

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Contents

ABSTRACT	1
INTRODUCTION	3
ASSISTED REPRODUCTIVE BIOTECHNOLOGY	6
ARTIFICIAL INSEMINATION	7
SEMEN COLLECTION	8
SEMEN CRYOPRESERVATION	13
OOCYTE CRYOPRESERVATION	25
OESTRUS SYNCHRONIZATION	28
IN VITRO PRODUCTION OF EMBRYOS	32
PREGNANCY DETERMINATION	40
CONCLUSION	40
SELECTED IMAGES OF ACTIVITIES	42
SELECTED PUBLICATIONS	46
REFERENCES	50
BIOGRAPHY	61
ACKNOWLEDGEMENTS	65
LIST OF INAUGURAL LECTURES	67

ABSTRACT

Successful livestock breeding until production of a healthy offspring is one of the key factors in ensuring sustainable animal production. The use of assisted reproductive biotechnology such as artificial insemination has always been practiced in ruminant sectors worldwide but its application in Malaysia is limited among farmers who are able to envisage the benefit of the technology in upgrading the genetic quality of their animals. The success of artificial insemination involves many factors such as semen collection, semen cryopreservation, handling of cryopreserved semen, insemination time and establishment of pregnancy. Therefore, this lecture dwells around research findings in the many processes in semen collection and cryopreservation which eventually improving the quality of semen prior to artificial insemination. It also discusses other aspects of livestock breeding such as oestrus synchronization and pregnancy diagnosis as well as in vitro embryo production.

INTRODUCTION

In all over the world, livestock production and the demand for livestock products continues to rise due to the steady growth of world's population which is expected to reach 10 billion by 2050. The demand for livestock products in Malaysia is also in the increasing trend. To date, Malaysia continue to meet its self-sufficiency level in two livestock namely pigs and poultry. In pigs, the self-sufficiency level is growing steadily at 99.24% (2011) to 100.51% (2011), while in poultry is at 113.68% (2001) to 128.14% (2011) (DVS, 2013).

On the other hand, the per capita consumption of livestock products is also continue to increase not only for pigs and poultry but also for other livestock products. This is evidence in the per capita consumption of beef, from 5.01 kg (2001) to 5.71 kg (2011) and, mutton/chevon, from 0.66 kg (2001) to 0.83 kg (2011) (DVS, 2013). Domestic farm animals, including cattle, sheep and goats, are important to the world economy. They are also very important livestock to smallholder farmers in developing countries such as Malaysia for their meat and milk. Like other countries, the ruminant population especially cattle and goats have been steadily increasing over the past ten years in Malaysia. The total population of cattle and goats in Malaysia was estimated at 855,543 and 523,800 heads in 2011 (DVS, 2013), respectively. This statistics is still far from fulfilling the requirement of the country and will certainly face with many challenges in satisfying the need of more than 32 million Malaysian in 2020. One of the strategies is to adopt and implement proper planned and assisted breeding in livestock particularly in the ruminant sector.

Malaysia: Self-Sufficiency in Livestock Products (%), 2001-2011

KOMODITI Commodity	WILAYAH Region	2001	2002	2003	2004	2005	2006	2007	2008	2009 ^P	2010 ^P	2011 ^F
DAGING LEMBU/ KERBAU Beef	Malaysia	16.06	17.89	18.49	17.95	21.15	21.78	23.50	25.38	26.98	28.65	29.02
	S. Malaysia	16.98	18.74	18.49	18.33	22.17	22.93	25.10	26.62	27.78	29.99	30.38
	Sabah Sarawak	17.32 4.96	12.66 7.00	18.49 18.49	21.28 8.07	7.52 13.30	19.97 6.90	7.45 6.29	9.38 9.03	20.03 10.79	11.46	11.62
DAGING KAMBING/BEBIRI Mutton	Malaysia	5.94	7.70	10.36	8.73	8.60	8.99	9.57	10.04	10.32	10.58	11.28
	S. Malaysia	5.68	7.65	10.53	8.73	8.67	9.04	9.60	10.10	10.39	10.67	11.35
	Sabah Sarawak	td 6.15	td 9.23	td 6.93	td 9.75	td 6.75	td 7.69	td 8.79	td 8.56	td 8.69	td 8.36	td 9.50
DAGING BABI Pork	Malaysia	99.24	98.68	99.32	99.66	98.82	98.87	96.91	96.61	96.88	101.69	100.51
	S. Malaysia	98.91	98.24	99.15	98.97	95.14	95.19	96.07	95.55	96.11	100.67	99.59
	Sabah Sarawak	99.89 101.18	99.96 101.06	100.63 99.91	100.00 102.43	99.97 100.00	102.63 104.76	99.99 100.27	99.98 101.37	100.78 101.37	102.14 107.30	104.56 104.61
DAGING AYAM/ITIK Poultry Meat	Malaysia	113.88	109.40	107.74	107.80	124.74	124.94	121.77	121.84	122.22	127.89	128.14
	S. Malaysia	66.10	112.22	110.85	110.12	131.15	132.45	128.25	127.41	126.24	132.08	132.20
	Sabah Sarawak	82.05 100.00	75.61 100.00	72.34 101.11	81.51 100.00	77.00 91.76	84.74 95.74	74.99 87.43	78.86 90.20	91.25 91.21	97.98 95.43	100.70 95.63
TELUR AYAM/ITIK Chicken/Duck Eggs	Malaysia	112.39	115.29	113.82	111.70	105.20	109.06	111.38	112.30	114.72	115.40	117.70
	S. Malaysia	114.78	119.03	116.68	124.61	108.25	109.76	115.30	110.77	117.49	118.17	120.53
	Sabah Sarawak	100.00 100.84	99.80 100.00	100.00 100.00	101.50 100.00	289.44 100.34	101.20 100.29	202.53 87.78	114.29 91.50	114.98 91.50	117.45 92.05	93.92
SUSU Milk	Malaysia	2.24	2.83	2.86	2.98	3.59	4.66	4.79	4.89	4.88	4.88	5.00
	S. Malaysia	2.46	2.30	2.44	2.56	3.85	4.00	3.75	4.40	4.37	4.36	4.47
	Sabah Sarawak	39.23 0.69	84.69 0.65	86.09 2.17	36.17 2.05	87.36 2.29	46.32 1.82	71.47 2.08	44.33 2.18	88.89 1.57	89.07 1.70	91.34 1.65

P : Sementara (Provisional) E : Anggaran (Estimate) td : Tidak maklumat (Not available)

(Source: Department of Veterinary Services Malaysia, 2013)

Malaysia: Per Capita Consumption of Livestock Products, 2001-2011

KOMODITI Commodity	WILAYAH Region	2001	2002	2003	2004	2005	2006	2007	2008	2009 ^P	2010 ^P	2011 ^E
DAGING LEMBU/KERBAU Beef (Kg)	Malaysia	5.01	5.06	5.18	5.81	5.32	5.49	5.48	5.43	5.60	5.75	5.71
	S. Malaysia	5.56	5.77	5.71	6.85	6.15	6.38	6.28	6.35	6.64	6.69	6.56
	Sabah	1.64	1.66	3.46	0.91	2.11	1.49	1.86	1.47	1.78	1.59	1.54
	Sarawak	4.38	3.01	2.59	2.81	1.51	2.78	3.00	2.42	2.53	2.59	2.59
DAGING KAMBING/BEBIRU Mutton (Kg)	Malaysia	0.66	0.60	0.50	0.59	0.65	0.67	0.68	0.70	0.75	0.80	0.83
	S. Malaysia	0.79	0.73	0.60	0.72	0.79	0.81	0.83	0.85	0.91	0.96	1.00
	Sabah	id	id	id	id	id	id	id	id	id	id	id
	Sarawak	0.31	0.22	0.27	0.19	0.28	0.28	0.29	0.30	0.32	0.34	0.36
DAGING BABI Pork (Kg)	Malaysia	7.75	7.97	7.67	7.86	8.63	8.40	7.60	7.28	7.62	8.15	7.80
	S. Malaysia	**19.38	**19.92	**19.19	**19.65	**21.58	**21.00	**19.00	**18.20	**19.06	**20.36	**19.50
	Sabah	**20.28	**20.76	**19.26	**19.73	**19.38	**21.52	**18.79	**18.52	**19.49	**20.86	**20.01
	Sarawak	3.30	3.00	2.90	4.30	4.50	4.20	3.20	3.50	2.45	2.72	2.46
DAGING AYAM/ITIK Poultry Meat (Kg)	Malaysia	12.53	11.95	14.31	**35.08	**33.23	**34.15	**35.20	**30.50	**31.84	**33.28	**33.41
	S. Malaysia	28.03	31.88	31.85	33.63	30.07	31.11	33.24	34.38	35.26	35.86	35.34
	Sabah	30.18	35.60	35.22	37.43	32.66	32.33	36.00	37.68	39.01	39.66	39.19
	Sarawak	16.90	17.54	1.82	19.13	21.12	23.80	26.84	26.10	26.67	27.08	26.80
TELUR AYAM/ITIK * Chicken/Duck Eggs (Egg)	Malaysia	251	248	251	253	260	267	271	280	290	301	299
	S. Malaysia	**15.04	**14.88	**15.08	**15.18	**15.60	**16.01	**16.26	**16.79	**17.38	**18.08	**17.92
	Sabah	262	250	261	265	278	279	279	294	294	306	319
	Sarawak	208	302	302	255	251	267	321	313	324	336	335
SUSU Milk (Kg)	Malaysia	56.07	51.73	51.08	50.84	34.26	36.63	39.27	41.67	45.74	48.61	48.06
	S. Malaysia	53.52	67.84	63.06	62.89	42.52	45.18	48.47	51.54	56.84	60.39	59.86
	Sabah	4.53	2.23	2.35	5.50	2.65	4.94	5.20	4.46	2.49	2.65	2.57
	Sarawak	6.13	6.36	1.46	1.29	1.13	1.17	1.20	1.33	1.55	1.64	1.63

P : Sementara (Provisional)
 E : Anggaran (Estimate)
 id : Tidak maklumat (Not available)
 ** : Berdasarkan kepada 40% bilangan penduduk bukan Islam
 *** : Berdasarkan kepada 40% non-Muslim
 * : Anggaran perata berat telur ayam/itik = 60 gram/ biji
 * : Estimated average weight of chicken/duck egg = 60 gm/egg

(Source: Department of Veterinary Services Malaysia, 2013)

Livestock Breeding: The Past, The Present and The Future

Malaysia: Livestock Population, 2007-2011

JENIS TERNAKAN <i>Livestock Type</i>	2007	2008	2009	2010	2011 ^E
KERBAU (<i>Buffalo</i>)					
S.Malaysia (<i>P. M'sia</i>)	80,931	79,661	74,979	72,799	71,913
Sabah	42,157	43,422	44,200	44,642 ^P	45,088
Sarawak	7,687	8,146	7,973	7,734 ^P	7,502
Jumlah (<i>Total</i>)	130,775	131,229	127,152	125,175	124,503
LEMBU (<i>Cattle</i>)					
S.Malaysia (<i>P. M'sia</i>)	772,323	779,877	786,317	761,796	778,189
Sabah	57,944 ^R	57,745 ^R	60,191 ^R	61,395 ^P	62,623
Sarawak	11,919 ^R	13,605 ^R	13,983	14,352 ^P	14,731
Jumlah (<i>Total</i>)	842,186	851,227	860,491	837,543	855,543
KAMBING (<i>Goat</i>)					
S.Malaysia (<i>P. M'sia</i>)	373,319	419,720	452,467	432,085	460,728
Sabah	45,742	45,742	47,110	47,779 ^P	48,457
Sarawak	9,202	12,018	14,656	14,635 ^P	14,615
Jumlah (<i>Total</i>)	428,263	477,480	514,233	494,499	523,800
BEBIRI (<i>Sheep</i>)					
S.Malaysia (<i>P. M'sia</i>)	122,106	125,931	130,723	118,300	117,108
Sabah	1,970	1,989	2,009	2,029 ^P	2,049
Sarawak	1,912	3,358 ^R	3,553	3,020 ^P	2,567
Jumlah (<i>Total</i>)	125,988	131,278	136,285	123,349	121,724
BABI (<i>Swine</i>)					
S.Malaysia (<i>P. M'sia</i>)	1,441,036	1,407,195	1,401,190	1,462,814	1,466,747
Sabah	82,200	105,075	83,972	77,926 ^P	72,315
Sarawak	496,881	476,619 ^R	346,146	339,569 ^P	333,117
Jumlah (<i>Total</i>)	2,020,117	1,988,889	1,831,308	1,880,309	1,872,179

P : Sementara (Provisional)

E : Anggaran (Estimate)

R : Semakan Semula (Revised)

(Source: Department of Veterinary Services Malaysia, 2013)

ASSISTED REPRODUCTIVE BIOTECHNOLOGY

Over the last two decades, various assisted reproductive biotechnology (ARB) are commonly applied in animal production to enhance their reproductive capacity, improve and preserve livestock genetics and develop new products such as transgenic or cloned animals.

The use of ARB has become increasingly widespread in livestock industry. The acceptability of cryopreservation of semen and embryo, artificial insemination, in vitro embryo production, and embryo transfer have led to establishment of many breeding organizations, semen and embryo banks, as well as sire stations. This is especially true in the dairy and beef cattle industry, where semen and embryo technological advancements have been the

most successful and widely used. However, the resulting quality of cryopreservation in many species is not similar to that of the initial specimen collected and face with many challenges.

ARTIFICIAL INSEMINATION

Assisted reproductive biotechnologies (ARB) are necessary to accelerate the efficiency of reproduction especially in ruminant. Amongst all ARBs, artificial insemination (AI) is the most effective technology for upgrading the breed and improving the reproductive rates in domestic animals. Artificial insemination has been used extensively in livestock production especially in cattle. It has also been used as a tool for genetic improvement programs, the introduction of specialized breeds, development of selected programs and progeny testing. Therefore, the use of AI serve as a better option for increasing the reproductive performance and upgrading of existing ruminant breed in this country.

Although natural mating is still preferred by many ruminant farmers, many farmers develop interest to breed their animals using AI and therefore good quality cryopreserved semen is needed to ensure the animal is pregnant following AI. This requires proper establishment of the current techniques of processing, cooling, packaging and freezing of semen which will enhance the subsequent viability and fertility of spermatozoa. Several factors many contribute to the low fertility with cryopreserved semen, which include cold-shock, osmotic stress and oxidative stress. All these are related to the procedures of cryopreservation that lead to spermatozoa damage, impairs its function and fertilizing potential.

In Malaysia, AI in cattle has been introduced since 1960's. Its widespread use is still faced with many challenges and are limited to farmers who are capable of seeing the advantage of using AI in upgrading their animals. One of the most important factors in

assuring the success of AI is the ability to produce good quality semen obtained from superior quality males and subsequently maintained either chilled or frozen thawed prior to AI. The basic principle of chilling or freezing is similar for spermatozoa of most mammalian species, but spermatozoa from different species may react differently due to their differences in morphology and certain biochemical constituents. It is worth mentioned that cryopreservation of goat semen differs from that of the other species, such as bull, boar, or ram. This is because the seminal plasma of goat's semen contains phospholipase secreted by the bulbourethral gland. It interacts with egg yolk resulting in coagulation of egg yolk media and hydrolyses lecithin to fatty acids and spermicidal lysolecithins (Leboeuf *et al.*, 2000). Hence, cryopreservation protocol developed for one species may not be ideal for spermatozoa of other species (Sundararaman and Edwin, 2008). Various researchers reported successful cryopreservation of goat spermatozoa but the fertility rate with chilled and frozen stored goat spermatozoa is variable. Many steps involve in the entire process of semen cryopreservation such as collection, washing, dilution, cooling, equilibration, freezing and thawing, and during which spermatozoa may eventually lose its ability to fertilize normally. Therefore, cryopreservation protocols for ruminant semen particularly goats raised in this country should be developed with the aim to maximize the quality and minimize the losses during cryopreservation.

SEMEN COLLECTION

As indicated earlier that semen must be collected, evaluated and cryopreserved prior to artificial insemination. Successful artificial insemination depends on many factors not only the exact time of insemination but also the quality of collected and cryopreserved semen. Several semen collection techniques such as using artificial

vagina and electro-ejaculation have been noted to produce good quality semen in livestock as well as wildlife animals.

It has been showed that semen can be collected safely from anesthetized lesser mouse deer using electro-ejaculation. The semen characteristics of this species were similar to those of semen collected by electro-ejaculation from other ruminants (Haron *et al.*, 2000). Apart from that, the morphology was also evaluated and measured. The spermatozoon in the lesser mouse deer is smaller than ram (Evans and Maxwell, 1987) and bull (Salisbury and Van Demark, 1985) with the head is relatively more round compared to the other ruminants (Prasetyaningtyas *et al.*, 2006).

In another study, it has been indicated that electro-ejaculation can be used to obtain semen from untrained (*Bos javanicus*) Bali bulls with 100% success rate and perhaps regarded as the preferred method since it requires relatively shorter time prior to collection. Electro-ejaculation also provide acceptable post-thawed semen quality that would permit faster reproduction and conservation of the Bali cattle (Sarsaifi *et al.*, 2013).

Percentage response, penile protrusion and semen emission of Bali bulls subjected to three semen collection methods

Parameters	Method of semen collection		
	EE (n = 25)	RM (n = 25)	RM + EE (n = 25)
No. of bulls responded to collection method (%)	25 (100) ^a	21 (84) ^b	24 (96) ^a
No. of bulls displayed penile protrusion (%)	24 (96) ^a	20 (80) ^b	23 (92) ^a
Time required for penile protrusion (s)	55.14 ± 1.22 ^b	80.08 ± 1.78 ^a	49.40 ± 0.28 ^b
Time required for semen emissions (s)	76.30 ± 1.95 ^b	116.77 ± 3.93 ^a	70.27 ± 0.67 ^b
No. of electrical stimuli required for penile protrusion	14.43 ± 0.76	NA	13.85 ± 1.92
No. of electrical stimuli required for semen emission	23.20 ± 0.97	NA	22.12 ± 2.31

NA, not applicable.

Mean values with different superscripts in the same row are significantly different at $p < 0.05$.

(Sarsaifi et al., 2013)

Semen parameters of Bali bulls subjected to three semen collection methods

Semen parameters	Method of semen collection (mean ± SEM)		
	EE	RM	RM + EE
Volume (ml)	6.98 ± 0.29 ^a	3.85 ± 0.19 ^b	6.97 ± 0.22 ^a
pH	7.04 ± 0.31 ^u	6.88 ± 0.37 ^b	7.05 ± 0.03 ^u
General motility (%)	74.44 ± 1.73	73.83 ± 1.46	74.52 ± 1.78
Viability (%)	72.54 ± 1.60	71.36 ± 2.85	71.04 ± 1.49
Sperm concentration (× 10 ⁶)	688.98 ± 18.3 ^b	831.55 ± 20.70 ^a	685.63 ± 14.67 ^b
Acrosome integrity (%)	73.73 ± 1.63	74.05 ± 0.91	72.81 ± 1.23
Normal morphology (%)	73.14 ± 1.24	71.50 ± 1.71	72.02 ± 1.30
Sperm head defects (%)	6.29 ± 0.34	6.02 ± 0.40	6.98 ± 0.42
Detached sperm heads (%)	3.67 ± 0.30	4.44 ± 0.31	4.33 ± 0.31
Sperm midpiece defects (%)	3.08 ± 0.28	3.41 ± 0.23	3.54 ± 0.46
Proximal cytoplasmic droplet (%)	3.94 ± 3.02	4.92 ± 3.09	4.85 ± 0.25
Tail abnormality (%)	9.90 ± 0.44	9.33 ± 0.76	10.33 ± 0.52

Means values in the same rows with different superscripts are significantly different at $p < 0.05$.

(Sarsaifi et al., 2013)

Effects of method of collection on acrosomal integrity and parameters calculated by CASA system for fresh and frozen-thawed semen in Bali bulls

Semen sample	Fresh		Frozen	
	Semen collection method			
	EE	RM	EE	RM
General motility (%)	77.43 ± 2.75	76.53 ± 2.18	63.80 ± 1.89 ^b	67.90 ± 0.53 ^{3a}
Progressive (%)	68.80 ± 2.36	67.25 ± 1.63	50.46 ± 1.99 ^b	56.73 ± 0.65 ^a
VAP (µm/s)	101.82 ± 1.28	103.14 ± 2.69	74.90 ± 1.38 ^b	80.20 ± 1.18 ^a
VSL (µm/s)	89.57 ± 1.59	90.27 ± 1.26	63.73 ± 1.61 ^b	67.88 ± 0.95 ^a
VCL (µm/s)	140.39 ± 3.22	141.62 ± 2.60	126.95 ± 2.16 ^b	132.37 ± 2.14 ^a
ALH (µm)	5.82 ± 0.15	6.17 ± 0.38	5.98 ± 0.13	5.84 ± 0.87
BCF (HZ)	28.46 ± 0.90	29.62 ± 0.67	24.29 ± 0.85	24.77 ± 1.18
STR (%)	87.35 ± 1.12	88.12 ± 1.06	86.72 ± 1.88	84.79 ± 1.19
LIN (%)	63.87 ± 0.84	64.07 ± 1.12	50.02 ± 0.98	51.30 ± 0.74
AI (%)	74.63 ± 1.54	75.21 ± 1.03	58.23 ± 0.94 ^b	62.06 ± 0.88 ^a

Data are means ± SEM. For each factor, different superscripts within a column differ significantly ($p < 0.05$).

RM, transrectal massage; EE, electro-ejaculation; VAP, velocity average path; VSL, velocity according to the straight path; LIN, linearity index; ALH, amplitude of lateral head displacement; BCF, beat cross frequency; STR, straightness; LIN, linearity and AI, percentage of spermatozoa with intact acrosome; CASA, computer-assisted semen analysis.

Means values in the same rows by semen type with different superscripts are significantly different ($p < 0.05$).

(Sarsaifi et al., 2013)

SEMEN CRYOPRESERVATION

Successful semen cryopreservation is one of the important factors for the application of reproductive techniques such as artificial insemination and in vitro embryo production which contribute to upgrade and increase animal production. Currently, many research in semen cryopreservation continue to improve existing procedure in order to establish better quality semen after thawing. Research in determination of appropriate extender including cryoprotectants, concentration of antioxidants and their method of addition, cooling rates and freezing method are some of the research areas that being conducted not only in livestock but also in wildlife animals.

However, cryopreservation causes ultrastructural, biochemical, and functional damages on spermatozoa due to the temperature changes resulting in decreased motility and viability. In addition, causes of reduced sperm motility are related to seminal plasma enzymes. Therefore, seminal plasma plays an important role in sperm survival during cryopreservation process (Salamon and Ritar, 1982).

The deterioration and toxic effect of the seminal plasma were observed when goat's semen was diluted with egg yolk or milk extender. Nowadays, these extenders are widely used for the frozen storage of small ruminant semen (Salamon and Maxwell, 2000). The presence of enzymes in the seminal plasma caused the harmful interactions between seminal plasma and egg yolk or milk which decreases sperm motility and movement quality by disruption of cell membrane (Pellicer-Rubio and Combamous, 1998), resulting in a loss of motility, membrane integrity and consequently low fertility rate (Upreti *et al.*, 1999).

Although removal of seminal plasma in small ruminant semen may be useful, several researchers however reported that this procedure may or may not have favourable effect on semen freezing

and thawing properties in goat semen (Kozdrowski *et al.*, 2007; Peterson *et al.*, 2007). Removal of seminal plasma is also a time consuming process that can damage the spermatozoa and need to be correctly performed. Therefore, previous research have used a variety of washing solutions and concentration regimes to find out the proper method for the improvement of cryopreserved semen quality. The centrifugation regimes and washing solutions used in buck semen cryopreservation varies from 400-2400 x g for 3-15 min with different washing solutions (Tuli and Holtz, 1994; Azeredo *et al.*, 2001; Peterson *et al.*, 2007; Kozdrowski *et al.*, 2007; Sariozkan *et al.*, 2010; Carvajal *et al.*, 2004).

Due to limited studies on evaluating the effects of washing solutions and centrifugation regime on Boer goat semen, several experiments were carried out with the objectives to analyze the effects of seminal plasma removal using two different extenders, three different washing solutions and different centrifugation regimes on the characteristics of goat semen.

The results indicated that the practical and beneficial effects can be obtained by removing seminal plasma through centrifugation (3000 x g for 3 min) with Tris-based extender as washing solution in Boer goat semen cryopreservation protocol. However, these results are based only on motility characteristics, and therefore, further fertility trials are required as the ultimate test of improved cryopreservation (Soe Win Naing *et al.*, 2011).

Effects of three different washing solutions on Boer goat semen characteristics

Before freezing		Progressive motility %	Intact acrosome %	Live spermatozoa %	Normal spermatozoa %
PBS	79.50 ± 1.17 ^a	85.30 ± 1.32	78.70 ± 1.45 ^a	93.40 ± 0.81	
NS	76.50 ± 1.50 ^b	84.20 ± 1.16	78.90 ± 1.27 ^b	91.40 ± 0.60	
TCG	84.50 ± 0.89 ^c	87.50 ± 1.43	83.20 ± 1.45 ^c	95.60 ± 0.58	
After thawing		Total motility %	Intact acrosome %	Live spermatozoa %	Normal spermatozoa %
PBS	54.50 ± 2.29 ^a	47.50 ± 1.11 ^a	67.70 ± 0.78 ^a	61.40 ± 0.95 ^a	88.70 ± 1.51
NS	51.50 ± 2.89 ^b	44.50 ± 1.74 ^b	66.10 ± 1.19 ^a	59.60 ± 1.31 ^a	87.90 ± 1.50
TCG	61.50 ± 2.24 ^c	54.50 ± 0.89 ^c	73.90 ± 0.66 ^b	68.50 ± 0.86 ^b	89.90 ± 0.89

Mean ± SEM within each column, means with different alphabetical superscripts (a,b,c) are significantly different at least as P<0.05 (ANOVA - Post hoc test). PBS = Phosphate Buffer Solution, NS = Normal Saline, TCG = Tris citric acid glucose extender

(Soe Win Naing *et al.*, 2011)

The procedure of semen cryopreservation includes several necessary steps such as temperature reduction, cellular dehydration, freezing and thawing. If it is not properly handle, all these procedures inevitable produce cold shock and oxidative attack on the sperm membrane, which subsequently decreased spermatozoa survival and fertilizing ability leading to death and also imposed negative effects on the preservation of semen for artificial insemination. Cold shock affecting spermatozoa is partly associated with oxidative attack and the generation of reactive oxygen species. Excessive production of reactive oxygen species during semen cryopreservation has been associated with reduced spermatozoa post-thaw motility, viability, membrane and acrosome integrity, antioxidant status and fertility.

As indicated earlier that the seminal plasma of goat semen is packed with many antioxidant components, so its removal will make spermatozoa more vulnerable due to the low concentrations of natural antioxidants. Therefore, it is more imperative to restore the normal level of antioxidants in semen by adding them in extenders during the semen cryopreservation process. This process also overcomes the detrimental effects of reactive oxygen species (Donoghue and Donoghue, 1997). The molecules of the antioxidant could reduce the impact of oxidative stress and thus improve semen quality after thawing. There are a number of antioxidants used for this purpose in many livestock species such as butylated hydroxytoluene (BHT), a synthetic phenolic antioxidant. It is a synthetic analogue of vitamin E that checks the auto-oxidation reaction by converting peroxy radicals to hydroperoxides. Butylated hydroxytoluene has been tested successfully to preserve liquid semen and minimize cold shock damage in several livestock such as turkey (Donoghue and Donoghue, 1997) stallions (Ball *et al.*, 2001), boars (Roca *et al.*, 2004), bulls (Shoae and Zamiri, 2008) and buffaloes (Ijaz *et al.*, 2009). In spite of these promising results, the

use of BHT in freezing extenders is not common and limited data is available with respect to its use in the goat semen cryopreservation.

Due to the limited data, further studies are required to obtain more information on the effectiveness of antioxidant capacity and to determine the most effective additives in the freezing of goat semen. Therefore a study was initiated to investigate the effect of various concentrations of BHT on sperm characteristics of Boer goat semen cryopreservation. The study also aims to identify the optimal concentration of BHT supplementation in Tris egg yolk based extender during cooling and freezing.

The results showed that addition of 2.0 mM butylated hydroxytoluene in Tris egg yolk based extender during cooling and freezing provide the optimal protection of Boer goat semen during cryopreservation (Memon *et al.*, 2011).

Rate of motility, membrane integrity, morphology, acrosome integrity and viability of Boer goat spermatozoa before freezing at various concentrations of butylated hydroxytoluene (BHT)

BHT (mM)	Motility (%)	Membrane integrity (%)	Morphology (%) ^m	Acrosome integrity (%)	Viability (%)
0.0	64.40 ± 0.90 ^a	67.80 ± 0.53 ^a	85.20 ± 0.65	68.90 ± 0.64 ^a	76.60 ± 0.67 ^a
0.5	65.90 ± 0.53 ^{ab}	69.90 ± 0.53 ^b	85.10 ± 0.66	71.30 ± 0.83 ^b	78.50 ± 0.50 ^{ab}
1.0	69.80 ± 0.73 ^c	71.70 ± 0.42 ^{cd}	85.50 ± 0.52	73.40 ± 0.92 ^b	78.60 ± 0.85 ^b
2.0	69.90 ± 0.55 ^c	72.20 ± 0.47 ^d	85.80 ± 0.47	73.70 ± 0.87 ^b	78.40 ± 0.52 ^{ab}
3.0	66.70 ± 0.82 ^b	70.70 ± 0.52 ^{ac}	84.80 ± 0.63	72.40 ± 0.56 ^b	76.70 ± 0.54 ^{ab}
P-values	<.0001	<.0001	0.7908	0.0004	0.0476

Values with different superscripts within column differ significantly at P<0.05; ^m no significant difference.

(Memon *et al.*, 2011)

Percentages of motility, membrane integrity, morphology, acrosome integrity and viability in post thaw Boer goat spermatozoa at various concentrations of butylated hydroxytoluene (BHT)

BHT (mM)	Motility (%)	Membrane integrity (%)	Morphology (%)	Acrosome integrity (%)	Viability (%)
0.0	57.10 ± 0.55 ^a	54.10 ± 0.95 ^a	80.30 ± 0.82 ^a	58.10 ± 0.60 ^a	69.70 ± 0.52 ^a
0.5	57.60 ± 0.72 ^a	58.20 ± 0.74 ^b	82.60 ± 0.54 ^b	57.90 ± 0.66 ^a	70.80 ± 0.51 ^{ab}
1.0	61.40 ± 0.69 ^b	59.90 ± 0.75 ^b	82.80 ± 0.73 ^b	61.10 ± 0.55 ^{ab}	71.60 ± 0.43 ^b
2.0	63.20 ± 0.53 ^c	59.40 ± 0.52 ^b	82.20 ± 0.66 ^{ab}	61.80 ± 0.53 ^c	71.70 ± 0.52 ^b
3.0	57.40 ± 0.56 ^a	55.40 ± 0.69 ^a	80.50 ± 0.48 ^a	60.00 ± 0.42 ^b	70.70 ± 0.42 ^{ab}
P-values	<.0001	<.0001	0.0191	<.0001	0.0356

Values with different superscripts within column differ significantly at P<0.05.

(Memon *et al.*, 2011)

The function of sugar in the extender for cryopreservation is to maintain the osmotic pressure of the diluents by inducing cell dehydration and less ice crystal formation into the spermatozoa. Apart from the function as energy source to support spermatozoa motility and movement, sugar has the ability to form a glass (vitrification) by depressing the membrane phase transition temperature of dry lipids. It also interacts with phospholipid membranes at low hydration and thus causes stabilization of the membranes (Aisen *et al.*, 2002; Hinch *et al.*, 2006).

Many researchers have studied the effect of sugar supplementation in semen extender on the quality of cryopreserved spermatozoa. Although glucose has been suggested to be more suitable sugar than fructose, lactose or raffinose in Tris-based media in ram semen (Salamon and Visser, 1972), but several reports indicated that there were no effect on post-thaw spermatozoa motility with different sugar addition (Abdelhakeam *et al.*, 1991; Molinia *et al.*, 1994). In contrast, trehalose, xylose and fructose significantly increased total active sperm rates compared to other sugars (glucose, sucrose, galactose, lactose, maltose and raffinose) in frozen thaw samples of dog spermatozoa (Yildiz *et al.*, 2000). A more recent study indicated that monosaccharide, especially fructose enhanced the better effect on semen quality than disaccharides and trisaccharides in red deer epididymal sperm cryopreservation (Fernández-Santos *et al.*, 2007).

Due to its remarkable stabilizing properties to protect protein and lipids membranes from degradation during the freeze-drying process, trehalose has been extensively used to improve spermatozoa quality in semen cryopreservation. Trehalose improved sperm membrane integrity, spermatozoa viability and post-thaw fertility in ram semen (Bucak and Tekin, 2007), mouse (Bayarad *et al.*, 1998), dog (Yamashiro *et al.*, 2007), and boar semen (Gutiérrez-Pérez *et al.*, 2009). However, due to the paucity of information on its effect

on goat spermatozoa, several studies were conducted to determine the effect of two monosaccharide and two disaccharide sugars on sperm characteristics in Boer goat semen cryopreservation.

The results of these studies indicated that the combination of glucose (69.38mM) with trehalose (198.24 mM) minimizes cell damage and increases number of viable spermatozoa following cryopreservation of Boer goat semen (Naing *et al.*, 2010).

Effect of sugar combination on semen characteristics of Boer goat pre-freezing and post-thawing

Pre-freezing	Progressive motility%	Intact acrosome%	Live spermatozoa%	Normal spermatozoa%
G	77.3 ± 2.2	87.3 ± 3.6	83.0 ± 1.5	92.4 ± 2.2
St	76.9 ± 2.1	89.2 ± 3.8	83.8 ± 2.1	93.1 ± 1.9
Ft	76.2 ± 1.6	87.2 ± 3.7	82.8 ± 1.7	93.4 ± 2.3
Gt	75.5 ± 1.9	88.8 ± 3.9	82.7 ± 1.6	93.0 ± 1.9
Post-thawing	Total motility%	Acrosome integrity%	Membrane integrity%	Live spermatozoa%
G	50.1 ± 2.1 ^a	58.5 ± 3.2 ^a	53.7 ± 1.9 ^a	50.6 ± 4.0 ^a
St	47.2 ± 2.6 ^a	63.2 ± 2.0 ^a	51.4 ± 1.5 ^a	55.7 ± 2.4 ^a
Ft	59.3 ± 1.7 ^b	60.3 ± 2.4 ^a	62.8 ± 1.6 ^b	51.9 ± 2.8 ^a
Gt	67.4 ± 1.4 ^c	75.1 ± 3.5 ^b	69.2 ± 1.4 ^c	66.4 ± 2.3 ^b

Mean ± SEM within each column, mean with different alphabetical superscripts are significantly different (ANOVA-post hoc test at P<0.05). G = glucose (control), St = combination of sucrose and trehalose, Ft = combination of fructose and trehalose, and Gt = combination of glucose and trehalose.

(Naing *et al.*, 2010)

Effect of different concentrations of trehalose on semen characteristics of Boer goat pre-freezing and post-thawing

Pre-freezing	Progressive motility%	Intact acrosome%	Live spermatozoa%	Normal spermatozoa%
T1	80.5 ± 1.6	85.7 ± 0.8	90.8 ± 0.5	94.5 ± 0.9
T2	78.5 ± 2.1	83.7 ± 0.8	90.8 ± 0.7	95.6 ± 0.6
T3	80.5 ± 1.7	84.5 ± 0.9	91.1 ± 0.7	95.4 ± 0.4
T4	81.5 ± 1.7	85.0 ± 0.8	91.6 ± 1.2	96.1 ± 0.3
T5	82.0 ± 1.7	86.3 ± 0.9	90.7 ± 0.7	94.4 ± 3.2

Post-thawing	Total motility%	Forward motility%	Acrosome integrity%	Membrane integrity%	Live spermatozoa%	Normal spermatozoa%
T1	50.6 ± 1.9 ^a	40.1 ± 2.1 ^a	62.9 ± 2.3 ^a	57.9 ± 2.2 ^a	56.9 ± 2.9 ^a	91.7 ± 1.5
T2	58.7 ± 2.5 ^{bc}	47.9 ± 1.9 ^b	67.6 ± 2.5 ^{ab}	64.6 ± 1.9 ^b	61.4 ± 3.6 ^{ab}	92.5 ± 1.4
T3	62.8 ± 1.7 ^c	52.6 ± 1.7 ^b	71.9 ± 3.2 ^b	68.8 ± 1.9 ^{bc}	65.2 ± 1.9 ^b	93.1 ± 1.6
T4	72.7 ± 1.2 ^d	64.2 ± 1.9 ^c	84.6 ± 2.2 ^c	72.4 ± 1.8 ^c	77.4 ± 1.2 ^c	93.4 ± 2.5
T5	52.7 ± 2.3 ^{ab}	40.5 ± 2.0 ^a	63.2 ± 1.1 ^a	57.6 ± 1.5 ^a	57.9 ± 2.3 ^a	91.1 ± 1.9

Mean ± SEM within each column, mean with different alphabetical superscripts are significantly different (ANOVA-post hoc test at $P < 0.05$). T1 = 33.04 mM trehalose, T2 = 49.59 mM trehalose, T3 = 66.08 mM trehalose, T4 = 198.24 mM trehalose, and T5 = 264.32 mM trehalose.

(Naing *et al.*, 2010)

Procedures related with cryopreservation produce cold shock and oxidative attack, which reduces the post-thaw quality of semen for artificial insemination (Bucak *et al.*, 2009). The plasma membrane of mammalian spermatozoa presents high concentrations of polyunsaturated fatty acids, which make it susceptible to free radicals induced peroxidative damage, especially following cryopreservation, with a subsequent loss of sperm functions (Lenzi *et al.*, 2002).

Supplementing cryopreservation media with antioxidants improved the quality of semen against free radicals-induced damage (Sariozkan *et al.*, 2009; Memon *et al.*, 2011). However, due to contradictory effect of antioxidant on spermatozoa freezing, an experiment was conducted to investigate the effect of different concentration of ascorbic acid supplementation on Boer goat semen cryopreservation.

The results concluded that chilled and post-thawed sperm quality (motility, membrane integrity, morphology, acrosome integrity and viability) of Boer goat improved when a Tris-based extender supplemented with 2.5–8.5 mg/mL ascorbic acid was used (Memon *et al.*, 2013).

Mean + SEM of motility, membrane integrity, morphology, acrosome integrity and viability of Boer goat spermatozoa (a) before and (b) after freezing at various concentrations of ascorbic acid

Ascorbic acid, mg/ml	Motility (%)	Membrane integrity (%)	Morphology (%)	Acrosome integrity (%)	Viability (%)
(a)					
Control	59.42 ± 2.10 ^c	54.86 ± 1.45 ^c	73.85 ± 1.26 ^b	52.00 ± 1.13 ^c	68.43 ± 1.67 ^c
2.5	70.43 ± 1.36 ^b	63.15 ± 0.93 ^b	79.15 ± 0.76 ^a	64.29 ± 1.26 ^b	77.15 ± 1.20 ^b
4.5	71.43 ± 1.42 ^b	64.58 ± 0.94 ^b	81.43 ± 0.99 ^a	64.43 ± 1.17 ^b	79.85 ± 1.28 ^{ab}
6.5	72.58 ± 2.22 ^{ab}	68.15 ± 0.93 ^a	82.57 ± 2.45 ^a	65.15 ± 0.79 ^b	81.14 ± 1.51 ^{ab}
8.5	76.86 ± 1.42 ^a	70.00 ± 0.48 ^a	82.72 ± 0.91 ^a	70.85 ± 0.67 ^a	82.00 ± 1.0 ^a
(b)					
Control	43.58 ± 0.92 ^d	42.15 ± 0.63 ^d	72.15 ± 1.40 ^b	43.15 ± 1.48 ^e	66.29 ± 0.80 ^e
2.5	52.15 ± 0.94 ^c	49.85 ± 0.96 ^c	72.29 ± 1.06 ^b	52.00 ± 1.02 ^b	67.00 ± 1.02 ^c
4.5	57.85 ± 1.01 ^b	54.00 ± 0.97 ^b	72.58 ± 0.71 ^b	54.00 ± 1.17 ^b	68.15 ± 1.01 ^{bc}
6.5	65.00 ± 2.18 ^a	58.85 ± 1.05 ^a	75.58 ± 0.64 ^b	60.86 ± 1.22 ^a	70.00 ± 0.75 ^b
8.5	65.71 ± 1.70 ^a	59.43 ± 1.60 ^a	79.29 ± 1.74 ^a	63.29 ± 0.94 ^a	73.58 ± 1.04 ^a

Values with different superscripts within column differ significantly at $p < 0.05$.

(Memon *et al.*, 2013)

OOCYTE CRYOPRESERVATION

As stated earlier that cryopreservation is a challenging biotechnology tool where results are dependent on size of cells, stage of gamete development, and species of animal. Similar to spermatozoa, cryopreservation of oocytes and embryos also confronted with these variations. These variations are directly related to the biochemical and biophysical properties of oocytes and embryos at each stage and the type of species. Amongst all gametes and embryos, oocytes especially at the germinal vesicle stage are the most difficult to cryopreserve. Low viability after cryopreservation may be due to special characteristic features of oocytes (Kuwayama, 2007). The presence of dense cumulus cells, response to hypertonic cryopreservation solutions, presence or absence of channels in oolemma for turnover of each cryoprotectant agent (CPA), and physical changes of the zona pellucida due to exposure to CPAs are some of the factors that can hamper high cryosurvivability of immature oocytes. Until recently, few studies on cryopreservation of immature bovine oocytes have been reported. Although live birth of normal calves have been reported after freezing/thawing (Suzuki *et al.*, 1996) and vitrification/warming of immature bovine oocytes (Vieira *et al.*, 2002), the results were not satisfactory. However, due to its simplicity, inexpensive, and practicability, it is expected that vitrification will eventually replace the expensive and time-consuming conventional freezing.

Source of damages to oocytes during cryopreservation processes may derived from simple fundamentals of cryobiology such as equilibration temperature during handling of samples, cooling and warming rates, and different methods of vitrification. Equilibration temperature may directly affect the influx and efflux of CPAs through zona pellucida and oolemma of oocytes. This may results in either better protection or chemotoxicity to the oocyte. The

effects of higher rate of cooling and warming processes have been well acknowledged. The lower the cooling and warming rates, the higher is the possibility of ice crystal formation. Different types of cryodevices have been tested for vitrification of samples and some vitrification methods have been developed over the years ago. Each method is a combination of a cryodevice and CPAs mixture. The emergence of cryodevices resulting in high cooling and warming rates led to the possibility of decreasing the concentration of vitrification solution. Therefore, lower toxicity and higher viability rates encouraged researchers to follow the same method for oocytes at different developmental stages and species (Kuwayama *et al.*, 2005; Zhou *et al.*, 2010). However, very few reports have been published on the above mentioned matters for cryopreservation of immature bovine oocytes. Therefore, the effects of different equilibration temperatures (32°, 37°, and 41°C) and cryodevices (open pulled straw, OPS; electron microscopy grid, EMG and cryotop) on viability of vitrified immature bovine oocytes were investigated.

The results indicated that oocytes equilibrated at 37°C had significantly higher maturation, cleavage, and blastocyst rates compared to that at 32°C and 41°C (Hajarian *et al.*, 2010). The results also showed that the in vitro viability of vitrified immature bovine oocytes cryopreserved using cryotop device was higher than open-pulled straw and electron microscopy grid (Hajarian *et al.*, 2011).

Polar body extrusion rate following in vitro maturation at different equilibration temperatures

Polar body rates	OPS (n = 49)	Cryotop (n = 58)	EMG (n = 77)	Control (n = 69)
Polar body +	10 (26%) ^c	24 (41%) ^b	24 (32%) ^c	48 (70%) ^a
Polar body -	27 (50%) ^a	26 (45%) ^a	39 (52%) ^a	20 (29%) ^b
Degenerated	12 (24%) ^a	8 (14%) ^b	14 (18%) ^{ab}	1 (1%) ^c

Data were pooled from 4 replicates; ^{a,c}Values with different superscripts in the same row are significantly different; (p<0.05; ANOVA and Duncan's test)

(Hajarjian *et al.*, 2010)

In vitro viability of vitrified-warmed immature bovine oocytes equilibrated at different temperatures

Treatment	No. of oocytes	Viability (%)	Oocytes Cleaved (%)	≥8 cell embryos (%)	Blastocyst (%)
32°C	96	76.32±2.18 ^{ab}	30.84±0.58 ^b	13.05±4.21 ^b	3.65±1.18 ^{ab}
37°C	104	87.95±1.66 ^a	38.77±0.76 ^a	21.13±2.82 ^a	6.45±0.54 ^a
41°C	94	70.73±2.30 ^b	28.95±1.20 ^b	10.71±3.44 ^b	1.94±1.24 ^b

^{a, b, c}: Values in the same column with different superscripts differ significantly (p<0.05)

(Hajarjian *et al.*, 2010)

Polar body extrusion rate following in vitro maturation in different cryodevices

Polar body rates	OPS (n = 49)	Cryotop (n = 58)	EMG (n = 77)	Control (n = 69)
Polar body +	10 (26%) ^c	24 (41%) ^b	24 (32%) ^c	48 (70%) ^a
Polar body -	27 (50%) ^a	26 (45%) ^a	39 (52%) ^a	20 (29%) ^b
Degenerated	12 (24%) ^a	8 (14%) ^b	14 (18%) ^{ab}	1 (1%) ^c

Data were pooled from 4 replicates; ^{a,c}Values with different superscripts in the same row are significantly different; (p<0.05; ANOVA and Duncan's test)

(Hajarjian *et al.*, 2011)

Nuclear maturation of vitrified immature bovine oocytes

Nuclear stage	OPS (n = 37)	Cryotop (n = 50)	EMG (n = 63)	Control (n = 68)
GV	15 (41%) ^a	8 (16%) ^c	16 (25%) ^b	4 (6.0%) ^d
GVBD-MI	6 (16%) ^a	10 (20%) ^a	13 (20%) ^a	2 (2.5%) ^b
MII	13 (35%) ^d	29 (58%) ^b	29 (46%) ^c	60 (88.0%) ^a
Unclassified	3 (7%)	3 (5%)	5 (7%)	2 (2.0%)

Data were pooled from 4 replicates; ^{a-d} Values with different superscripts in the same row are significantly different ($p < 0.05$)

(Hajarian *et al.*, 2011)

OESTRUS SYNCHRONIZATION

In the tropical countries, animal production is described as multipurpose, in which they are being used for milking, meat, clothing, fertilizer, fuel, draft power and as a form of currency. For cattle production, the main challenges causing decreased reproductive efficiency are associated with long calving interval and delayed first oestrus postpartum due to the high incidence of postpartum anestrus. The slow recovery of reproductive performance during postpartum is a limitation to the success of reproductive management programs. Therefore, synchronization and resynchronization of oestrus as well as artificial insemination could be used to increase reproductive performance and improve calving interval.

In Malaysia, ruminant production has recorded poor growth rates and presently the national requirements for beef, goat meat and mutton are largely met by imports. The Department of Veterinary Services statistical analysis reported that the consumption of beef has increased from 120,270 metric tonnes in 2001 to over 168,273 metric tonnes in 2011 with the increment of per capita consumption of 5.1 in 2001 to 5.71 in 2011 (DVS, 2013). With the increment of the demand, steps should be taken to propagate the number of

animals in order to reduce the loss in revenue through importation bills.

The main objective in animal production is to provide efficient and effective management systems. Maintaining good reproductive performance is part of these effective systems and it can be achieved by implementing proper reproductive biotechnologies. Some of the reproductive biotechnologies that can be used to increase reproductive efficiency include oestrus synchronization, artificial insemination and semen cryopreservation.

Synchronization of oestrus in cattle can facilitate the use of AI by reducing the time needed for the detection of oestrus compared to cattle entering oestrus spontaneously. It is also considered as an effective means of increasing the proportion of females that become pregnant early with shorter calving seasons and produce uniform calves. It was reported that up to 85% of cattle enter oestrus between 36 and 60 hours after being synchronized with progestin release subcutaneous implants or intravaginal progesterone releasing inserts (Diskin *et al.*, 2002). It has also been reported that over 90% of cattle can be induced to come into oestrus within 24 hours (Cavalieri *et al.*, 2004).

Synchronization of oestrus in cattle not only allows one to predict the time of oestrus with reasonable accuracy but also helps the management in which a proper breeding programme may be planned and organized. Synchronization of oestrus also reduces the time required for detection of oestrus as well as facilitates for timed artificial insemination without detection of oestrus. Successful synchronization, followed by breeding will also improve pregnancy rates in beef cattle. To date, there are many methods of estrous synchronization; therefore, a study was conducted to determine the efficacy of estrous synchronization methods between intravaginal devices, i.e. controlled internal drug releasing device

(CIDR) versus progesterone releasing intravaginal device (PRID) in Kedah-Kelantan cows.

Results indicated that 18/24 (75.0%) cows showed standing heat following synchronization and majority of the cows that showed oestrus (11/18, 61.1%) were within 72 hours after removal of CIDR or PRID. Results also showed that cows treated with CIDR (91.7%; 11/12) had significantly better response to oestrus compared with PRID (58.3%; 7/12) (Wahid *et al.*, 2001). In conclusion, CIDR is more efficient than PRID in terms of estrous synchronization in Kedah-Kelantan cows.

In order to attain a year round calving interval, management of estrous cycle in a herd of cows is very critical and this can be achieved by implementing oestrus synchronization (Cavalieri *et al.*, 2008). However, resynchronization of oestrus and insemination in the non-pregnant cows after a first unsuccessful synchronization are utilized to reduce variations in inter-estrus interval. This procedure has been reported to improve and maintain reproductive efficiency (Eagles *et al.*, 2001; Stevenson *et al.*, 2003; McDougall and Loeffler, 2004). Furthermore, following the first synchronized oestrus and artificial insemination, cows can also be resynchronized for the subsequent second and third estrous cycles which subsequently helped to improve the reproductive performance of cows (Cavalieri *et al.*, 2000; Cavalieri and Macmillan, 2002). A resynchronization protocol which involves placing an intra-vaginal device containing progesterone for 12-15 days after the first insemination for 7-8 days, combined with an intramuscular injection of 0.5-1.0 mg oestradiol benzoate at the time of intra-vaginal device insertion and another injection of equal dose within 24-48 h after intra-vaginal device removal was reported to enhance pregnancy rate (Cavalieri *et al.*, 2000, 2004, 2008). These treatments consistently increased the synchrony of return to oestrus in non-pregnant cattle (Van Cleeff *et*

al., 1996; Stevenson *et al.*, 2003). However in CIDR-treated cattle, the initial pregnancy rate (Chenault *et al.*, 2003) or the pregnancy rate after resynchronization (Stevenson *et al.*, 2003) was reduced. Prostaglandin F2 alpha (PGF2a) and its synthetic analogues have been used to control the estrous cycle in cattle (Wright and Malmo, 1992). Nevertheless, the effectiveness of PGF2alpha to synchronize oestrus is dependent upon the presence of a responsive corpus luteum. Calving interval of 13.7 and 13.5 months have been reported in the Canada (Lucy, 2001) and the Netherlands (Fatehi and Schaeffer, 2003), respectively. Slow resumption of reproductive cycle during the postpartum period remains a problem in cattle raised in tropical countries like Indonesia and Malaysia with the period to first postpartum oestrus reported to be about 4.5 months (Yimer *et al.*, 2010).

Since many synchronization programmes have been used to facilitate oestrus detection rate in beef cattle but with varying degrees of success, therefore, a study was conducted to depict oestrus response and pregnancy rate of cows following synchronization and resynchronization with CIDR as well as between groups of cows resynchronized with CIDR and PGF2alpha. This study revealed a similar effect of both initial synchronization and re-synchronization with CIDR on Brangus cows in terms of the proportion of cows that exhibit oestrus (Malik *et al.*, 2011). It also showed that both CIDR and PGF2a protocols for oestrus resynchronization in postpartum Brangus cows have indicated a similar proportion oestrus response and pregnancy rates.

Percentage of estrus response and pregnancy in synchronization and resynchronization using CIDR and PGF2 α

Items	Total population	No. of cows mounting (%)	Cows of pregnant	Pregnancy (%) based on	
				Estrus response	Total sample
Initial (synchronization)	140	94/140 (67.1)	41	39/94 (41.4)	29.2
Resynchronization (CIDR)	49	40/49 (81.6)	15	15/40 (37.5)	30.6
Resynchronization (PGF α)	50	35/50 (70.0)	14	11/35 (31.5)	28.0

Non significant between resynchronization using CIDR and PGF α methods

(Malik *et al.*, 2011)

IN VITRO PRODUCTION OF EMBRYOS

In vitro production of embryos provides opportunities in many areas such as cryopreservation of oocytes and embryos, embryo transfer and cloning. Currently, it is well known facts that pre-implantation embryos can be produced via totally in vitro procedures, i.e. in vitro maturation (IVM), in vitro fertilization (IVF) and in vitro culture (IVC) prior to transfer to the recipient. Due to its vast number of applications, these procedures provide almost endless research opportunities in the field of in vitro production of embryos. To date, it has been indicated that approximately 15% of bovine embryos are produced by in vitro embryo production (IVEP) throughout the world (Mapletoft and Hasler, 2005).

The importance of cumulus cells for in vitro fertilization of matured oocytes is still a controversy among researchers. It is believed that there are some beneficial roles of cumulus cells on in vitro fertilization leading to higher cleavage rate and higher yield of blastocysts. However, in some earlier studies, the presence of cumulus cells surrounding the oocyte is not necessary for a successful fertilization (Behalova and Greve, 1993), and some other researchers have observed that cumulus cells interfere with sperm penetration (Hawk *et al.*, 1992). Therefore, a study was conducted to determine the effect of cumulus cells removal from

in vitro matured oocytes just before insemination on cleavage rate of bovine embryos. The result of the study suggests that intact-cumulus cells improved significantly the cleavage rate following *in vitro* fertilization of bovine oocytes, leading to higher cleavage rates (Sianturi *et al.*, 2001). The presence of intact cumulus cells provide a capacitation-inducing mechanism and facilitate the interaction between spermatozoa and zona pellucida (Cox *et al.*, 1993). It was also reported that the cumulus cells secrete heparin-like glycosaminoglycans which may render a supportive role in capacitation and fertilization and exert a specific effect on sperm motility and acrosome reaction (Ball *et al.*, 1983).

Effect of cumulus cells removal on cleavage rate

Types of oocytes	Numbers of oocytes inseminated	Numbers of oocytes cleaved (%)
Cumulus-intact	361	257 (71.2) ^a
Denuded	224	107 (47.8) ^b

Data were pooled from 4 replicates

^{a,b} Values with different superscripts in the same column are significantly different (P<0.01)

(Sianturi *et al.*, 2001)

Most of the reported studies in vitrification of oocytes and/or embryos focus on basics of cryobiology such as increasing cooling/warming rates (Morato *et al.*, 2008), vitrification solution ingredients (Yamada *et al.*, 2007) and temperature of equilibration solution (Hajarian *et al.*, 2010). Although, the previously studies resulted in more favorable results, still the trend of vitrification of immature bovine oocytes is not satisfactory enough. Besides the importance of cryobiological factors, developmental stage of

immature oocyte may play significant role not only for in vitro embryo production (IVEP) system but also for the vitrification outcome. Almost as a routine part of IVEP system activities, immature oocytes are selected based on morphology of oocyte and its surrounding cells (Hawk and Wall, 1994; Hazeleger *et al.*, 1995). It has been shown that a portion of morphologically selected oocytes are still in growing phase and not yet ready for maturation step (Rodriguez-Gonzalez *et al.*, 2002; Alm *et al.*, 2005). The pool of immature oocytes can be separated effectively using brilliant cresyl blue (BCB) stain test (Rodriguez-Gonzalez *et al.*, 2002; Manjunatha *et al.*, 2007). The BCB test is non-invasive and measures glucose-6-phosphate dehydrogenase (G6PDH) activity. G6PDH is a protein synthesized in growing immature oocytes and converts BCB stain to colorless. Conversely, grown oocyte cytoplasm which has lower levels of G6PDH remains blue in color (BCB+) because there is no reduction for BCB to a colorless compound (Bhojwani *et al.*, 2007; Ishizaki *et al.*, 2009). Based on previous studies, capability of BCB+ oocytes to develop to the blastocyst stage is higher than BCB-oocytes (Alm *et al.*, 2005; Manjunatha *et al.*, 2007; Opiela *et al.*, 2010) even after somatic cell nuclear transfer (Bhojwani *et al.*, 2007). This competency of BCB+ oocytes has been demonstrated on molecular and subcellular level as well (Tomer *et al.*, 2008). In addition, the BCB test also selected a significantly higher number of competent oocytes in heifers for in vitro embryo production (Pujol *et al.*, 2004). Majority of the genes upregulated in BCB+ oocytes are related to cell cycle, transcription and protein biosynthesis regulation (Tomer *et al.*, 2008). On top of that, BCB- oocytes had lower transcript level of genes involved in mitochondrial biosynthesis (Opiela *et al.*, 2010). So far however, no research has been found that studied the consequence of selection of developmentally competent oocytes on cryopreservation results.

Therefore, a study was conducted with the objective to evaluate the BCB test before vitrification and therefore enhance the maturity rate after vitrification of immature bovine oocyte.

The results of the study indicated that selection of immature oocytes using BCB stain was effective and could be used before cryopreservation of immature oocytes to improve the in vitro fertilization rate (Hajarian Hadi *et al.*, 2010).

Polar body (PB) extrusion rate of treatment groups after vitrification

Groups	N	PB+	PB-	Degenerated
Control	72	49.0 (36/72) ^a	51.0 (36/72) ^f	0 ^f
BCB+	71	23.9 (17/71) ^b	68.67 (49/71) ^{ab}	7.4 (5/71) ^b
BCB-	68	17.5 (12/68) ^b	60.5 (41/68) ^{bc}	22.0 (15/68) ^a
Vitrified-control	75	18.6 (14/75) ^b	75.1 (56/75) ^a	6.2 (5/75) ^{bc}

Data were pooled from 6 replicates, ^{a-d} Values with different superscripts in the same column are significantly different (p<0.05)

(Hajarian Hadi *et al.*, 2010)

Nuclear maturation rate of treatment groups after vitrification

Groups	N	Unclassified	GV	GVBD-MI	MII
Control	72	6.2 (4/72)	1.1 (1/72) ^f	5.7 (4/72) ^f	87.0 (63/72) ^a
BCB+	66	7.6 (5/66)	19.8 (13/66) ^b	21.1 (14/66) ^{ab}	51.58 (34/66) ^b
BCB-	53	8.6 (5/53)	46.6 (24/53) ^a	17.0 (9/53) ^b	27.9 (15/53) ^d
Vitrified-control	70	7.1 (5/70)	28.4 (20/70) ^b	21.4 (17/70) ^a	40.3 (28/70) ^f

Data were pooled from 6 replicates, ^{a-d} Values with different superscripts in the same column are significantly different (p<0.05)

(Hajarian Hadi *et al.*, 2010)

In recent years, in vitro embryo production (IVEP) systems have been acknowledged to increase the rate of transferable embryos in bovine (Galli *et al.*, 2004; Spicer *et al.*, 2007) and the final objective in IVEP is the production of transferable embryos and

birth of healthy offspring. To achieve these objectives, every steps in IVEP have to be efficient. Although pre-implantation embryos may develop after in vitro culture, obvious differences exist between their developmental rates compared to those developed in vivo. For instance, approximately 60-80% of in vivo matured bovine oocytes are competent to reach to the metaphase II stage while only 25-40% of in vitro matured oocytes reach to this stage (Blondin *et al.*, 2002; Dieleman *et al.*, 2002; Avery *et al.*, 2003). Failure to fertilize and develop to the blastocyst stage following in vitro procedures may indicate the lack of vital factors in the in vitro media which normally are available in vivo. Therefore, it is necessary to modify the media and conditions to support higher percentage of embryo development in vitro.

Several factors such as, hormones, proteins and growth factors supplemented to the culture medium may have crucial roles on the outcome of IVEP. It has been shown that insulin plays a crucial role for growth of variety of cells in the in vitro culture. Insulin bind to cell surface receptor and has been demonstrated that it stimulates glucose and amino acid uptake and protein synthesis (Harvey and Kaye, 1990; Kane *et al.*, 1997) of mouse embryos (Kaye and Harvey, 1995). Several studies have indicated that insulin increases the in vitro oocyte maturation and development of human (Dashtizad *et al.*, 2003), mice (Demeestere *et al.*, 2004) and porcine embryos (Lee *et al.*, 2005). Insulin receptor has also been detected in all stages of bovine embryos (Makarevich and Markkula, 2002). However, the results of using insulin are controversial for in vitro bovine embryo production and need more investigation. Therefore, several experiments were designed to study the effect of different concentration of insulin on bovine IVEP.

The results demonstrated that insulin promotes in vitro bovine embryos production. Supplementation of 10 µg/mL insulin

Abd. Wahid Haron

in maturation medium, exerted beneficial effects on nuclear maturation, cleavage and subsequent bovine embryo development *in vitro*. Furthermore, addition of insulin (10 $\mu\text{g}/\text{mL}$) to the culture medium showed positive effect on bovine embryo development to morula stage and slightly enhanced hatchability of produced blastocysts (Dashtizad *et al.*, 2010).

Effect of different concentration of insulin in IVM media on in vitro maturation of bovine oocytes and subsequent embryo development

Pre-freezing		Progressive motility%	Intact acrosome%	Live spermatozoa%	Normal spermatozoa%	
T1	80.5 ± 1.6	85.7 ± 0.8	90.8 ± 0.5	94.5 ± 0.9		
T2	78.5 ± 2.1	83.7 ± 0.8	90.8 ± 0.7	95.6 ± 0.6		
T3	80.5 ± 1.7	84.5 ± 0.9	91.1 ± 0.7	95.4 ± 0.4		
T4	81.5 ± 1.7	85.0 ± 0.8	91.6 ± 1.2	96.1 ± 0.3		
T5	82.0 ± 1.7	86.3 ± 0.9	90.7 ± 0.7	94.4 ± 3.2		
Post-thawing		Total motility%	Acrosome integrity%	Membrane integrity%	Live spermatozoa%	Normal spermatozoa%
T1	50.6 ± 1.9 ^a	40.1 ± 2.1 ^a	62.9 ± 2.3 ^a	57.9 ± 2.2 ^a	56.9 ± 2.9 ^a	91.7 ± 1.5
T2	58.7 ± 2.5 ^{bc}	47.9 ± 1.9 ^b	67.6 ± 2.5 ^{ab}	64.6 ± 1.9 ^b	61.4 ± 3.6 ^{ab}	92.5 ± 1.4
T3	62.8 ± 1.7 ^c	52.6 ± 1.7 ^b	71.9 ± 3.2 ^b	68.8 ± 1.9 ^{bc}	65.2 ± 1.9 ^b	93.1 ± 1.6
T4	72.7 ± 1.2 ^d	64.2 ± 1.9 ^c	84.6 ± 2.2 ^c	72.4 ± 1.8 ^c	77.4 ± 1.2 ^c	93.4 ± 2.5
T5	52.7 ± 2.3 ^{ab}	40.5 ± 2.0 ^a	63.2 ± 1.1 ^a	57.6 ± 1.5 ^a	57.9 ± 2.3 ^a	91.1 ± 1.9

Mean ± SEM within each column, mean with different alphabetical superscripts are significantly different (ANOVA–post hoc test at P < 0.05). T1 = 33.04 mM trehalose, T2 = 49.59 mM trehalose, T3 = 66.08 mM trehalose, T4 = 198.24 mM trehalose, and T5 = 264.32 mM trehalose.

(Dashtizad *et al.*, 2010)

Effect of insulin supplementation at different concentrations in IVC media on in vitro development of bovine embryo

Insulin concentration ($\mu\text{g mL}^{-1}$)	No. of cultured oocytes	Mean % of cleaved oocytes \pm SEM (n)	Mean % of morula oocytes \pm SEM (n)	Mean % of blastocyst \pm SEM (n)	Mean % of hatched blastocyst \pm SEM (n)
0 (Control)	213	78.86 \pm 2.79 ^{cd} (168/213)	60.55 \pm 2.56 ^{cd} (129/213)	38.99 \pm 2.77 ^a (83/213)	12.23 \pm 3.02 ^{ab} (26/213)
1	212	86.38 \pm 2.98 ^b (183/212)	74.60 \pm 3.25 ^b (158/212)	37.70 \pm 2.07 ^a (80/212)	10.32 \pm 3.06 ^{ab} (22/212)
10	201	90.58 \pm 2.02 ^a (182/201)	79.66 \pm 3.86 ^a (160/201)	35.89 \pm 3.44 ^{ab} (72/201)	14.49 \pm 3.72 ^a (29/201)
20	206	82.04 \pm 3.70 ^c (169/206)	63.13 \pm 3.25 ^c (130/206)	30.63 \pm 3.87 ^c (63/206)	8.77 \pm 2.75 ^b (18/206)
50	223	75.38 \pm 3.12 ^d (168/223)	58.31 \pm 3.50 ^d (130/223)	32.83 \pm 3.22 ^{bc} (73/223)	10.44 \pm 3.61 ^{ab} (22/223)

Data were pooled from 6 replicates. ^{a, b, c, d} Values with different superscripts in the same column are significantly different ($p < 0.05$; ANOVA and Duncan's test)

(Dashtizad et al., 2010)

PREGNANCY DETERMINATION

One of the most important aspect in livestock production is the ability to determine the pregnancy status of the animals post-breeding. The ability to determine pregnancy at the earliest time enables the management in planning for the subsequent activities such as separation from non-pregnant animals, improve feeding regime and preparing the animals for parturition. Early determination of pregnancy also allows the management to investigate and/or remove unproductive animals so that unnecessary feeding wastage can be reduced leading to more profit obtained per animal.

With the advancement of technology, it is now possible to determine pregnancy in livestock at the earliest possible after breeding. The use of ultrasound scanning technique, pregnancy can be determined in less than 1 month in most ruminant species. This technique is not only could be used in livestock but also in wildlife.

CONCLUSION

The use of assisted reproductive biotechnology and combined with improve management in animal production is definitely important and has been applied in many countries. These combination provide opportunities to increase animal production in order to meet the increasing demand of animal products.

As a take home message, several issues have been identified and could be exploited in order to improve the ruminant industry in Malaysia. They are:

- It is now possible to produce pre-implantation embryos via totally in vitro procedures, which are in vitro maturation, in vitro fertilization and in vitro culture.

- The vast biodiversity of wildlife in this country provides many opportunities and must be utilized to improve the livestock industry.
- Semen, oocytes and embryo cryopreservation techniques are available to support the process of conservation and propagation in animals.
- Molecular techniques are available to assist in determining the genetic quality of animal breed prior to the actual breeding.
- Advanced reproductive biotechnologies such as oestrus synchronization, artificial insemination and pregnancy detection should be used as part of management activities in livestock production particularly in the ruminant sector.

Our journey to achieve the self-sufficiency level in ruminant products in Malaysia is still far although many efforts are geared toward fulfilling the requirements. A concerted effort from government agencies especially Department of Veterinary Services, Malaysian Agriculture Research and Development Institute, universities as well as the private sectors especially the ruminant farmers will eventually produce more wholesome “Made in Malaysia” livestock products to meet the ever increasing demand from consumers.

SELECTED IMAGES OF ACTIVITIES

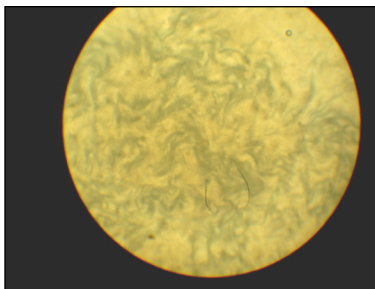


Figure 1 Semen wave pattern.
X40

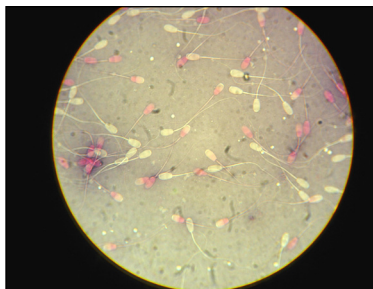


Figure 2 Stained spermatozoa
X400



Figure 3 Semen collection in
barking deer



Figure 4 Semen collection in
porcupines



Figure 5 Semen collection in
mouse deer



Figure 6 Estrus synchronization
using CIDR in goats



Figure 7 Transcervical artificial insemination in goats



Figure 8 Laparoscopic artificial insemination in goats



Figure 9 Large animal surgery class



Figure 10 Artificial insemination training for veterinary officers



Figure 11 Embryo recovery and transfer in goats



Figure 12 Embryo collection and evaluation in goats

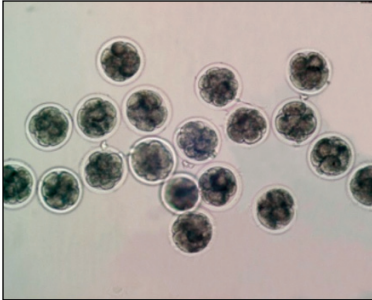


Figure 13 In vitro produced cattle embryos



Figure 14 Caesarean section in a goat



Figure 15 Pregnancy diagnosis in goats



Figure 16 Pregnancy diagnosis in horses



Figure 17 Pregnancy diagnosis in barking deer



Figure 18 Pregnancy diagnosis in elephant



Figure 19 Pregnancy diagnosis in porcupines



Figure 20 Ectopic pregnancy in sheep



Figure 21 Caesarean section in a cow



Figure 22 Caesarean section in a cow



Figure 23 Uterine prolapsed in a cow



Figure 24 Uterine prolapsed in a cow

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BIOGRAPHY

Born on May 17, 1963 in Batu Pahat, Johor, I enrolled in primary school from 1971-1976 and later continued at the secondary school from 1977-1982. From 1983 to 1988, I pursued my study for the degree of Doctor of Veterinary Medicine at Universiti Putra Malaysia. After working as a research assistant for about a year, I was offered a tutorship. From 1991-1993, I pursue my study in PhD in Animal Reproduction at the University College Dublin, Republic of Ireland under the supervision of eminent professor, Prof. Ian Gordon. During my 3-year study there, I have published 18 scientific papers in local as well as international journals and proceedings.

In 1993, I was appointed as a lecturer at the Faculty of Veterinary Medicine, Universiti Putra Malaysia. I was awarded with several research grants such as short-term and Intensification of Research in Priority Area (IRPA) projects, focusing on the use of assisted reproductive technologies in domestic ruminants.

In the beginning of this millennium, I was appointed as a member of the Asian Rhino Specialist Group, in collaboration with the International Rhino Foundation (IRF) and the Department of Wildlife and National Parks Malaysia (PERHILITAN), to specially stimulate the effort in breeding Sumatran Rhinoceros in captivity. This research was initiate to pilot a research in establishing an enzyme immunoassay technique for rhino fecal progesterone metabolite as well as improve captive rhino breeding.

Since I joined this Faculty, I have attended more than 50 courses or workshops, either as organizing committee member, invited speaker and participant. Apart from publishing papers in journals and proceedings, I am also a co-author of two chapters in the well-known textbook “Reproduction in Farm Animals - 7th. Edition” catering for mainly undergraduate students in reproductive biology,

veterinary medicine, and animal sciences. I have contributed many veterinary topics in Bahasa Malaysia for the *Ensiklopedia Sains dan Teknologi* (Science and Technology Encyclopaedia).

In April 1999, I was involved in the preparation for MS ISO 9001 certification as one of the steering committee. As a result of that, in 2000, Faculty of Veterinary Medicine, Universiti Putra Malaysia was awarded the certification covering a comprehensive eight core businesses. This achievement is the first faculty among Malaysia's public universities to achieve such award.

In 2000, I was awarded an Outstanding Excellence Service Award by Universiti Putra Malaysia. Subsequently, I was promoted to an Associate Professor in July 2001 and on 1st February 2002, I was appointed as Head, Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine UPM. Subsequently, on 1st July 2003, I was appointed as Deputy Dean of Academic and Student Affairs of the faculty.

On 29th October 2007, I was appointed as Deputy Dean in charge on the University Veterinary Hospital (UVH) of the faculty. I was appointed as Animal Biotechnology Consultant to ar-Raudhah Bio-Tech Farm Sdn Bhd, a BioNexus status company dealing with small ruminant management and biotechnology activities. In 2007, together with Innovation and Commercialization Centre, UPM, I manage to establish a spin-off company, Putra Al-Mawashi Genetics Sdn Bhd (PALM-Gen). The company successfully secured RM500,000 grant under Malaysian Technology Development Corporation (MTDC) focusing on commercialization of cattle frozen semen and services.

In 2010, I was the main author of the book “Reproduksi dan Peranian beradas Ternakan” published by UPM Press. This book focused on basic information on livestock reproduction and artificial insemination. It was targeted to not only those in the secondary

school diploma level but also farmer who are interested to seek in depth on animal reproduction.

On 1st January 2012, I was appointed as the Director of Taman Pertanian Universiti (TPU) (University Agriculture Park), one of the service provider centers in UPM providing and maintaining facilities not only in teaching and research but also looking at the opportunities for income generation of the university.

To date, I have supervised 19 PhD and 13 Masters students either as main or co-supervisor. I have also published more than 120 publications in the form of books, chapter in books, research articles in journals and proceedings as well as presented my research achievements in national and international conferences.

These output and achievements have provided me with input, energy, enthusiasm and commitment to continue doing greater career opportunities especially in teaching, research and post-graduate supervision. It also serves as a platform for a greater and more challenging leadership responsibility in the future.

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LIST OF INAUGURAL LECTURES

1. Prof. Dr. Sulaiman M. Yassin
The Challenge to Communication Research in Extension
22 July 1989
2. Prof. Ir. Abang Abdullah Abang Ali
Indigenous Materials and Technology for Low Cost Housing
30 August 1990
3. Prof. Dr. Abdul Rahman Abdul Razak
Plant Parasitic Nematodes, Lesser Known Pests of Agricultural Crops
30 January 1993
4. Prof. Dr. Mohamed Suleiman
Numerical Solution of Ordinary Differential Equations: A Historical Perspective
11 December 1993
5. Prof. Dr. Mohd. Ariff Hussein
Changing Roles of Agricultural Economics
5 March 1994
6. Prof. Dr. Mohd. Ismail Ahmad
Marketing Management: Prospects and Challenges for Agriculture
6 April 1994
7. Prof. Dr. Mohamed Mahyuddin Mohd. Dahan
The Changing Demand for Livestock Products
20 April 1994
8. Prof. Dr. Ruth Kiew
Plant Taxonomy, Biodiversity and Conservation
11 May 1994
9. Prof. Ir. Dr. Mohd. Zohadie Bardaie
Engineering Technological Developments Propelling Agriculture into the 21st Century
28 May 1994
10. Prof. Dr. Shamsuddin Jusop
Rock, Mineral and Soil
18 June 1994

Livestock Breeding: The Past, The Present and The Future

11. Prof. Dr. Abdul Salam Abdullah
Natural Toxicants Affecting Animal Health and Production
29 June 1994
12. Prof. Dr. Mohd. Yusof Hussein
Pest Control: A Challenge in Applied Ecology
9 July 1994
13. Prof. Dr. Kapt. Mohd. Ibrahim Haji Mohamed
Managing Challenges in Fisheries Development through Science and Technology
23 July 1994
14. Prof. Dr. Hj. Amat Juhari Moain
Sejarah Keagungan Bahasa Melayu
6 Ogos 1994
15. Prof. Dr. Law Ah Theem
Oil Pollution in the Malaysian Seas
24 September 1994
16. Prof. Dr. Md. Nordin Hj. Lajis
Fine Chemicals from Biological Resources: The Wealth from Nature
21 January 1995
17. Prof. Dr. Sheikh Omar Abdul Rahman
Health, Disease and Death in Creatures Great and Small
25 February 1995
18. Prof. Dr. Mohamed Shariff Mohamed Din
Fish Health: An Odyssey through the Asia - Pacific Region
25 March 1995
19. Prof. Dr. Tengku Azmi Tengku Ibrahim
Chromosome Distribution and Production Performance of Water Buffaloes
6 May 1995
20. Prof. Dr. Abdul Hamid Mahmood
Bahasa Melayu sebagai Bahasa Ilmu- Cabaran dan Harapan
10 Jun 1995

Abd. Wahid Haron

21. Prof. Dr. Rahim Md. Sail
Extension Education for Industrialising Malaysia: Trends, Priorities and Emerging Issues
22 July 1995
22. Prof. Dr. Nik Muhammad Nik Abd. Majid
The Diminishing Tropical Rain Forest: Causes, Symptoms and Cure
19 August 1995
23. Prof. Dr. Ang Kok Jee
The Evolution of an Environmentally Friendly Hatchery Technology for Udang Galah, the King of Freshwater Prawns and a Glimpse into the Future of Aquaculture in the 21st Century
14 October 1995
24. Prof. Dr. Sharifuddin Haji Abdul Hamid
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Fish Processing and Preservation: Recent Advances and Future Directions
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10 February 1996
27. Prof. Dr. Mohamed Ismail Abdul Karim
Microbial Fermentation and Utilization of Agricultural Bioresources and Wastes in Malaysia
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28. Prof. Dr. Wan Sulaiman Wan Harun
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29. Prof. Dr. Abdul Aziz Abdul Rahman
Sustained Growth and Sustainable Development: Is there a Trade-Off 1 or Malaysia
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30. Prof. Dr. Chew Tek Ann
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