Journal of Fisheries & Livestock Production

Maneewan, J Fisheries Livest Prod 2013, 1:3
DOI: 10.4172/2332-2608.1000106

Review Article Open Access

Artificial Breeding: IVM/IVF/IVC/ET/Cloning

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Abstract

Large Ruminants in Tropical Environment, they are buffaloes and cattle. In Thailand, as an area of hot climate and high humidity, the animals were subjected to adapt themselves not only temperature but even more harder in adaptation to humidity. Environment interaction among the nutrition plants and biological responses on temperature and humidity contributed greatly to depress the genetic expression. Production of large ruminant is, therefore, in tropical environment mainly focused on reproduction and health care. Natural selection was resulted in well performance but low reproductive rate. According to author's career as a chemist and biochemist she has been much concentrating on biological regulation for enhancing fertility and growth with co-incidentally better health care. The three aspects of achievement of fertility, growth and health within one injection may be called "Three in One that is Mato-Matid technology" However, Mato-Matid technology did provide a great deal of prime biological responses positively to several reproductive technologies in which some of them may be regarded as artificial breeding like IVM/IVF/IVC/ET/Cloning.

Introduction

Reproductive technologies in farm animals that created in Thailand during the 30 years of author's career may be summarized as follows:

Early pregnancy diagnosis (EPD)

EPD using progesterone (P_4) test to detect non pregnant animals in cattle and buffaloes was well established in milk and plasma determination. The P_4 test was simplified as a kit test which is available for farm testing.

Mato-Matid technology

The technology was illustrated as a hardware using active immunization against anabolic substances and as software conceptualizing the optimum condition of man, animal and plant to live together for reproducing the clean products, low cost and self-supporting.

Artificial breeding

Artificial breeding before implantation was generally described in the world. The consequential steps in laboratory were described in terms of; *in vitro* maturation (IVM), *in vitro* fertilization (IVF) and *in vitro* culture (IVC) then the grading embryos were transferred to the recipients. The recent development of cloning from somatic cells in mammal was a breakthrough of science technology in bringing out the artificial breeding for mass reproduction.

Altogether the said three reproductive technologies were established by the author in tropical condition of the developing country liked Thailand. Almost the techniques in these technologies were published and disseminated throughout the world by regular training and bilateral technical assistance.

The important advances in artificial breeding and the control of buffalo and cattle reproduction have been made during the last three decades (1971-2000) in Thailand under the author's direction. The first decade (1971-1980) was emphasized on improving the efficiency of artificial insemination using progesterone (P_4) test. Methodology of developing P_4 test in plasma for buffalo and in milk for dairy cattle were intensively established. P_4 test has been operated as a routine for early pregnancy diagnosis in farm level as well as disseminated to participants from many countries all over the world.

The second decade (1981-1990), there was a need to solve the problems of sub fertility, the delay of ovarian resumption and the incidence of endometritis. The ongoing activity of endocrine regulation was turned key to use active immunization against testosterone that enable to develop "Matid" an agent to overcome the said problems. In order to increase the productivity of young breeders, the young stocks were improved by enhancing the calf growth rate both in buffalo and cattle, using an agent called "Mato"; an active immunization against pregnenolone. It is, therefore, Mato-Matid technology has been established since then.

Cloning

The artificial breeding on IVM/IVF/IVC and ET was systematically developed since last two decades (1981-2000). It was a unique stepwise to up the laboratory in associated with training the personnel and also providing the routine services on farm level. For the third decade (1991-2000), the success of embryo transfer in dairy herds and *in vitro* embryo production in cattle, buffalo and pig was ready to establish as a regular program. It was capable to be topped up by cloning in the year 1998.

Historic record of Thailand in artificial breeding, the twining female calves, whom born by embryos from two donor cows and transferred to one recipient in 8 September, 1986 named "Jum" and "Jim", was a historic achievement of Thailand in ET. Recently in the year 2000 on the 6th of March, the first cloning female calf named "Ing" in twining with an AI male calf named "Oun" were born from a recipient dairy cow named "Oil" was also becoming a historic achievement of Thailand, a brief information was recorded as follows.

The success of first cloning female calf "Ing" was reproduced from the fusion of somatic cells "ear cell" of a donor heifer Figure 1 Brangus (Brahman x Angus) aged 13 months old with the enucleated mature oocyte collected from slaughterhouse. The cloning blastocyst of 7 days was transferred to a Holstein Friesian recipient that already inseminated 7 days before. She was under the Matid program to enhance fertility and delivered the twining calves of Ing (female cloning) and Oun (male AI). They were in 276 days gestation.

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Received May 22, 2013; **Accepted** June 22, 2013; **Published** June 28, 2013

Citation: Maneewan K (2013) Artificial Breeding: IVM/IVF/IVC/ET/Cloning. J Fisheries Livest Prod 1: 106. doi:10.4172/2332-2608.1000106

Figure 1: First cloning heifer ***Ing*** (Brangus, picture 1-a) in twining with AI male OUN (picture 1-b), they were born on 6 March 2000, picture with Professor Maneewan Kamonpatana took on 4th March 2003 when ***Ing*** was nearly 3 years old.

Group 1	Milking cow	Dry cows-Post partum cows that waiting for AI
Group 2	Inseminated cows	waiting for EPD and Pregnancy Confirmation
Group 3	Confirm Pregnancy	waiting for calving
Group 4	Replacement heifers	starting from yearling up to first conception
Group 5	Female calves	rearing up to 12 months of age before moving into Group 4

Table 1: Database of semi manual-computerized program to monitoring the management of concurrent moving from 5 grouping of the herd.

The second female cloning calf (donor cells from 6 months age of female Holstein Friesian) was lost during delivery due to the over body weight of the calf (47 kg).

The third female cloning calf named "*Nikolaus*" was born on the 3rd of April 2001. She was reproduced from the fusion of somatic cells "ear cell" of a donor American Brahman which born in Thailand and having the age of 6 months old with the enucleated mature oocytes collected from slaughterhouse. The cloning blastocyst of 7 days was transferred to a Brangus recipient. The recipient was under Matid program to enhancing fertility and she was pregnant for 301 days gestation.

Cloning of buffaloes and pig are in progress. Several somatic cell types were introduced to the program. The appropriated artificial breeding has been assessed and providing to animals belonging to the farmers, it was the dynamic arrangement that enable the new technology to be seen and accepted directly by the users.

Economic Optimization of Reproductive Performance

Reproductive Performance of a dairy herd affects *profitability* of the farm.

Reproductive inefficiency reduces milk yield and numbers of calves born, replacements production and may increase the cost of veterinary services; it also affects the culling rate.

Insemination, treatment, and culling decisions represent the dynamic of dairy herd management that ultimate affecting *profitability*.

To make rational decisions, the farmer needs a valid estimation of the future profitability of each cow, accounting for factors including age, production level, lactation stage, and pregnancy status and disease history.

Reproductive Management Program (RMP) can be used for optimizing a sequence of interrelated decisions. RMP is a dynamic program that developed for optimizing breeding and replacement decisions in dairy herd; as illustrated in the database of semi manual-computerized program to monitoring the management of concurrent moving from 5 grouping of the herd Table 1.

It is necessary to convince the farmers to cooperate and keeping up in collecting data manually then put in computer, RMP was developed gradually then the prediction of 10 years plan ahead was proposed to generate the estimate herd of 100 milking cows and project to 10 years operation. This program was well accepted by the farmers that operated 50-100 milking cows.

In recent years, the integration of five technologies has been implemented.

Profitability of using integrated technologies of

1. EPD
2. RMP
3. Mato – Matid Technology
4. CMN block
5. IVM/IVF/IVC/ET/Cloning

Profitability was shown in comparison with non using of the said five technologies, particular Mato-Matid technology. The farmers realized the advantages of employing the said technologies that evidences by investigation of monthly incomes in the farm.

It is our ultimate goal to monitoring RMP under tropical condition to increase the percentage of shorter calving interval.

The ideal calving interval of 12 months can be obtained only the optimal replacement was operated in the farms.

Optimal Replacement and Breeding Decision

There are 5 factors that contributed to breeding decision in order to replace the breeding cows optimally.

(1) High milk yield cows were necessary to take care of energy balance and they should conceive not later than 90 days post partum.

(2) Genetic merit cows must be assessed for milk yields, lactation periods and numbers of insemination per conception.

(3) Sire selection using high pedigree of frozen semen must be planned properly in breeding decision.

(4) Replacement heifers must be well kept the record of genetic merit, both from dams and sire selection.

(5) Reproductive disorder in the milking cows that retained over year after mediated by Mato-Matid technology should be culled.

Mato-Matid technology

The swamp buffaloes of Thailand as well as the other countries in China and Indochina have been in existence for over 6,000 years. Fossilized bones of swamp buffaloes excavated at H8-Mu-Tu, in Yu-Yao county, Ningbo City, Zhejiang province were from distinct successive earth's crust strata, the oldest materials being lowest in the sequence. Fossils in the lowest stratum were 9,000 to 10,000 years old: those in the upper stratum were dated at approximately 7,000 years of age [1]. In recent times the swamp buffalo in Thailand is drastically reduced. The problems can be crystallized in to its low reproductively under traditional rural practices. The female buffaloes have poor ovarian functions due to poor nutrition; this also gives rise to higher rate of abortion and high calf mortality [2]. What's more this low reproductively is further exacerbated by the lack of mating bulls. The reason for this is in

tradition practices the 3-4 years old bulls are castrated making them docile and conducive to rural domestic and farming use.

The challenge during the author early career was how to improve the buffaloes fertility and reduce the rate of calf loss.

Early researches concluded that the abuse of buffaloes under socio-economic depression lead to malnutrition and sub fertility of these durable animals [3].

Tests were initiated to normalize the hormones of the female buffaloes, several compounds of sex steroids conjugated to protein or gelatin were developed as an active immunogen. These compounds, ranging from Androstenedione, Estradiol, Estrone, Testosterone and DHEA, are injected into the female buffaloes.

Finally, testosterone immunogen was found to be suitable. These injections averaged 1.8-3.2 injections, depending on the state of sub fertility that the female buffaloes were needed. The immunogen produced excellent result increasing the average calving rate from 18-21% to over 70%.

A new immunogen of pregnenolone conjugated with a protein or gelatin called "Mato" (pronounced MATO as in go) was incidentally formed by testing in male buffaloes. The substance was successfully used in both male and female. Those animals that were inoculated become fast growing and fertile and were also resistant to foot and mouth disease.

"Mato-immunogen" produced an excellent result. The bulls' body weight reached the average puberty target in greater quantity than those not injected with this "Mato immunogen".

The term "Mato" is a Thai words meaning "come and be bigger" other words come and get this injection and be bigger. While the term "Matid" (pronounced-MATEED) means "come and be fertile" - come and get this injection and be fertile [4-7].

These "Mato-Matid" technology were extended to other farm animals such as cattle, pigs, sheep, goat, duck and chicken with satisfactory results (see references).

From these 30 year, experiments and observations the author postulated that the interrelationship between man, animal and plants are constantly, coexisting by modulation each other's so optimal equation exists. Since the modern society is materialistic with the aim of maximization of economic gains, this equilibrium will be upsetted with the dire consequence to our environment and ultimately to ourselves. The mato-matid technology provides us with the lesson of optimization rather than maximization to re-establish the disequilibrium between man, animal, plant for his animal and for himself and his environment. The optimum technology must have low cost, be a clean product and self supporting or at least sustainable as illustrated in Figure 2 [8-14].

How Mato-immunogen is working? It may be postulated that it works as a Complex Network Interaction. Mato is working as a mediator to start the chain of reaction in the whole body with no limited action. A dynamic event was already shown in the magnificent attribute of enhancing growth, improve reproduction and pathogenic resistance when Mato was presented to the body and acting as a trigger to start the immune function. The action of Mato may possible be a chain reaction which gets through the immune system. The cellular mechanism of action of Mato was suggested to take up as a Complex Network Interaction that enable cytokines to bridge the endocrine system through the nervous system thus these system are working dynamically in the anabolic correspondence. It may be proposed that

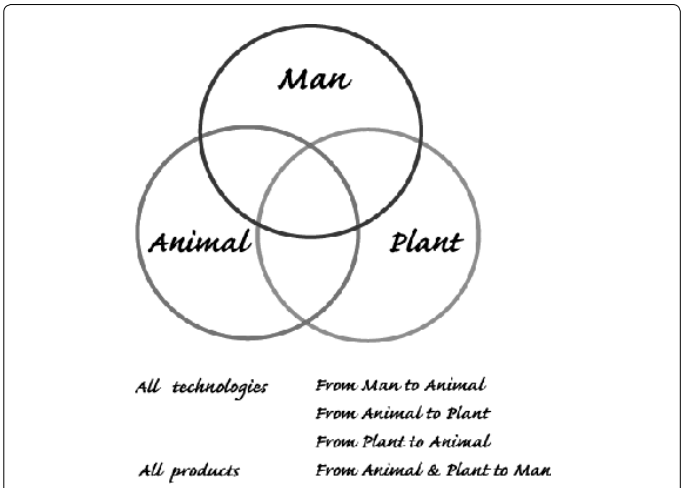

Figure 2: Optimal integration of Man, Animal and Plant to keep art of living together in the manner of peaceful life for all.

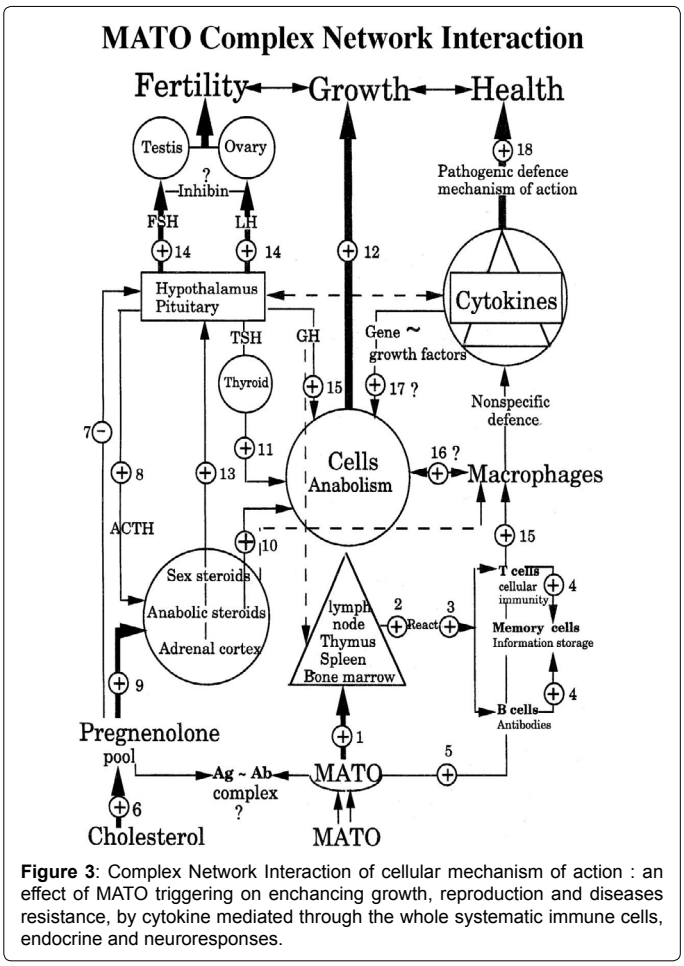

Figure 3: Complex Network Interaction of cellular mechanism of action : an effect of MATO triggering on enchancing growth, reproduction and diseases resistance, by cytokine mediated through the whole systematic immune cells, endocrine and neuroresponses.

such chain reaction is a powerful mechanism to maintain the vitality and health of the whole body Figure 3. On top of this view, the meaning of life may be defined as *"life is an active biological chain reactivities in the body"*.

Originated from "Discovery of Mato for Enhancing Animal Productivity and Reproductivity" by Professor M. Kamonpatana, from Animal and Plant Technology, Proceedings of the seminar held at Sichuan

Agricultural University, Yaan, Sichuan, People's Republic of China during 6-10 May 1996 under the framework of Technical Exchange between Chulalongkorn University (CU) and Sichuan Agricultural University (SAU) during 1993-1996, page 202 (pages 199-218).

The author would like to suggest that in nutrient requirement and health care, it may be worthwhile to exploit their uses through the concept of Mato-Matid technology means using vaccination to substitute oral treatment.

Mato-Matid Technology consisted of two components as 1) **Hardware** of synthesized immunogens for vaccination and 2) **Software** of lesson to manage the optimal condition; that allowed man, animal and plant to living together in the manner of peaceful life for all [15-18].

Conclusion of Moderate Way In Order To Achieve the Productivity

Low cost; clean products; self supporting:

1. It is a long term for developing country to get everything in order.
2. Benefit towards Profit needed to Balance all the time. The success both from humanness and output of technologies was based on the dynamic management under the integration of man, technical approach and economical acceptance due to time by time.

IVM/IVF/IVC/ET/Cloning: The laboratories of IVM/IVF/IVC for embryo transfer and cloning in both cattle and buffalo has been established at Chulalongkorn Unviersity (CU) since 1978. The CU laboratory has been contributed to train the scientists and cooperate with the other institution to develop the IVM/IVF/IVC laboratories. Such cooperation as well as collaboration was gradually developed year by year. By the time, there are more than 5 laboratories are under the way of research and development in IVM/IVF/IVC for livestock.

The achievement of the laboratory at CU under the author's guidance may briefly describe as follows: in Tables 2-5. The key point of the tables showed that early life of buffalo before implantation was different from cattle.

It is interesting to investigate further that buffalo oocytes or buffalo somatic cells which a component can contribute to a faster developing of cloned embryos to reach blastocysts than cattle components.

It is not yet to conclude that buffalo cloning was getting many

Available Knowledge	*Cattle*	*Buffalo*
1. Artificial Insemination (AI) Activities		
- frozen semen	Worldwide use and industrial support	Developing, non industry
- % First conception	30 – 40 %	< 20 %
- % Conception per year	60 – 95 %	50 %
2. Heart of AI		
- Ovary & oocytes development	Bigger ovary, oocytes protude on the surface of ovary which easily detect	Smaller ovary, oocytes embedded in the ovary which difficult to detect
- Heat detection & appropiate insemination	80 – 100 % clear oestrous symptom	50 – 60 % silent oestrous symptom
- Nature of the cow	Regular ovulation, cycle 20 days	Irregular ovulation, cycle 18 – 32 days
- Pregnancy	280 ± 10 days	320 ± 10 days
3. Preparation of Recipients	Much easier for preparation	Difficult to prepare and get small number
4. *In vitro* Fertilization (IVF)		
- No. of oocytes / ovary	5 - 25	1- 10
- Maturation of oocytes	60 – 90 %	12 – 75 %
- IVF	60 – 90 %	5 – 75 %
- IVC to Blastocyst	32 – 49 %	0.5 – 20 %
5. Embryo Freezing	Developed & worldwide uses	Developing and difficulties
6. Embryo Transfer		
% embryo grade 1-2 from ;	-	
Donors (by flushing)	≥ 80	≥ 70
IVF (by M II oocytes)	≥ 40	≥ 15
Cloning (by M II oocytes)	≥ 30	≥ 20
7. Pregnancy rate (%) of the recipients at 60 days of ET		
- Embryos from Donors	≃ 60	≃ 20
- Embryos from IVF	≃ 50	< 10
- Embryo from Cloning	≃ 35	Not available
8. Calving rate(%) from pregnant recipients which rectum palpated at 90 days of ET		
- Embryos from Donors	≃ 95	Not detected
- Embryos from IVF	≃ 80	Not detected
- Embryos from Cloning	≃ 50	Not available
9. % Normal neonatal growth		
- Embryos from Donors	≥ 95	Not detected
- Embryos from IVF	≥ 95	Not detected
- Embryos from Cloning	≤ 70	Not available

Table 2: Process of Life Synthesis by Cloning In Cattle and Buffalo; Achievement in Thailand.

Items of comparison	*Cattle (Ear fibroblasts)*	*Buffalo (Granulosa cells and fetal fibroblasts)*
No. of ovaries	219	709
No. of oocytes cultured	898 (4.1/ovary)	986 (1.4/ovary)
No. of MII oocytes	704 (78.4%)	575 (58.3%)
No. of success enucleation	658 (93.5%)	542 (94.2%)
No. of fusion	577	522
No. of fused	523 (90.6%)	454 (86.9%)
No. of cultured	523	454
No. of cleaved	470 (89.8%)	397 (87.4%)
No. of 8-cells	343 (65.5%)	294 (64.7%)
No. of morulae	249 (47.6%)	116 (25.5%)
No. of blastocysts	211 (40.3%)	94 (20.7%)
1. AI + ET	61 recipients (68 embryos)	Non transferred
- Concieve	22 (36%)	
- Aborted during 100-150 days	2 (0.9%)	
- Calving cloned calf (276 days gestation)	1 (0.45%)	
- Calving calves from AI	19 (86.4%)	
2. Non-AI and ET	27 recipients (36 embryos)	
- Pregnant	10 (37.0%)	
- Aborted	5 (50.0%)	
50-60 days before term	2	
20 days before term	1	
Calving at 273 days gestation (died)	1	
Calving at 301 days gestation	1	

Table 3: Summary of Trials of Cloned Embryos during 1998-2000: A Comparison between Cattle and Swamp Buffalo Using Donors Cell of Ear Fibroblasts and Granulosa Cells and Fetal Fibroblasts respectively.

difficulties than cloning in cattle. It is eventually to develop the suitable techniques for buffalo. It might be hard to conclude that IVM/IVF/IVC/ET/cloning in buffalo and cattle is the same. They are similar and actually they are difference. Particular techniques for buffalo must be developed to succeed the satisfactory of reproductive targets [19]. Scientists who work with buffalo should realize such differences and work hard to achieve the targets of buffalo production.

Cloning: Why in cows: As the author is a chemist and in fact a biochemist by training from the Faculty of Science, Chulalongkorn University, she would like to pay her deepest gratitude to all passed away teachers and senior teachers for their contribution in transferring their scientific minds to her during her education period 1962-1967. Such a scientific mind does brought up her career to establish the laboratory in Bioscience at the Faculty of Veterinary Science, Chulalongkorn University by her own talent and great effort to run the laboratory throughout her working period within government official service during 1969-2001.

Beside many ongoing activities of the said Laboratory that operated in Thailand as well as the collaboration work outside Thailand; Cloning is one of the activities that is interested by the public. She would like to declare **"Cloning: Why in Cows."** The rational capacity behind the title was the availability of her appropriate set up both in laboratory and on farming services that resulted to achieve the first cloning female calf in Thailand.

The first cloning female calf was born on 6 March 2000 and named ING; she was served by artificial insemination on the 22 November 2002. Hopefully she will deliver her first calf around September 2003. The second cloning female calf was lost during delivery on the 6 March 2001. The third cloning female calf named *Nikolaus* was born on 3 April 2001, she is healthy and having the body weight of 556 kg on 13 March 2003 at the age of 1 year 11 month and 10 days. She was served by the

	8 Cells					*Morulae*					Blastocyts			
	SE of CT		*SE of SB*			*SE of CT*		*SE of SB*			*SE of CT*		*SE of SB*	
	2	(53.20)	9	(48.45)			-	3	(110.00)			-	3	(134.00)
	1	(53.20)	4	(50.04)		1	(108.45)	1	(107.50)		1	(132.45)	1	(146.45)
	5	(53.35)	9	(51.02)		1	(111.00)	6	(114.00)		1	(133.00)	5	(149.00)
	8	(54.45)	10	(51.03)			(102.45)	3	(123.35)			-	3	(147.35)
	4	(55.10)	9	(51.10)		1	(127.10)	2	(116.10)		1	(149.00)	2	(140.55)
	13	(55.20)	4	(53.20)			(103.00)	4	(126.20)				4	(155.00)
	9	(55.43)	10	(53.45)		4	(119.00)	7	(104.15)		4	(183.00)	5	(140.05)
	4	(56.20)	8	(54.33)		2	(134.00)		-			-		-
	4	(56.35)	7	(55.00)		1	(120.00)	2	(108.00)		1	(167.00)	1	(165.00)
	8	(56.45)	7	(55.01)		4	(105.45)	2	(120.00)		1	(132.10)	2	(143.10)
	4	(57.37)	16	(57.30)		2	(130.45)	8	(110.00)		2	(178.37)	6	(136/00)
	6	(58.02)	11	(58.08)		4	(106.02)	4	(144.00)		2	(151.50)	4	(159.00)
	1	(58.10)	13	(58.35)		1	(121.00)	4	(101.00)		1	(149.00)	3	(136.00)
	1	(59.30)	6	(62.06)		1	(133.00)	1	(104.00)		1	(165.00)	1	(120.00)
	2	(60.10)		-		1	(105.05)		-			-	1	(163.00)
Range	1-13		4-16			1-4		1-8			1-4		1-6	
	(53.20-59.30)		**(48.45-62.06)**			**(102.45-134.00)**		**(101.00-144.00)**			**(132.10-183.00)**		**(120.00-165.00)**	
X±SD	5.00 ± 3.51		8.79 ± 3.26			1.69 ± 1.44		3.62 ± 2.22			1.50 ± 0.97		3.08 ± 1.66	
	(55.84 ± 1.92)		**(54.17 ± 3.79)**			**(117.00 ± 11.65)**		**(114.48 ±11.73)**			**(154.04 ±18.76)**		**(143.96 ± 11.77)**	

Table 4: A Comparison of Hours in Co-Culture Clone Embryos to reach the Stages of 8 Cells, Morulae and Blastocyst between Somatic Ear Cells of Cattle (Se of Ct) Fused into Cattle Oocytes and Somatic Ears Cells of Swamp Buffaloes (Se Of Sb) Fused Into Swamp Buffalo Oocytes.

	8 Cells					*Morulae*					*Blastocyts*			
	SE of CT x SB oocytes		*SE of SB x CT oocytes*			*SE of CT x SB oocytes*		*SE of SB x CT oocytes*			*SE of CT x SB oocytes*		*SE of SB x CT oocytes*	
	2	(53.30)	4	(53.26)		2	(125.30)	1	(133.44)		1	(152.00)	0	(165.00)
	1	(54.10)	1	(57.45)		1	(136.00)	1	(115.12)			-	0	(157.30)
	3	(55.21)	5	(58.55)		2	(130.20)	1	(104.11)		1	(152.20)	0	(160.10)
	4	(55.38)				2	(130.54)				1	(161.30)		
	1	(56.32)				1	(106.00)				1	(130.00)		
	2	(57.58)				1	(106.58)				1	(145.00)		
	3	(59.45)				1	(125.10)							
Range	1-4		1-5			1-2		1			1		-	
	(53.30-59.45)		**(53.26-58.55)**			**(106.00-136.00)**		**(104.11-133.44)**			**(130.00-161.30)**		**(157.30-165.00)**	
X±SD	2.29 ± 1.11		3.33 ± 2.08			1.43 ± 0.53		1			1		-	
	(55.91 ± 2.10)		**(56.42 ± 2.79)**			**(122.82 ± 11.87)**		**(117.56 ±14.82)**			**(148.10 ±11.66)**		**(160.80 ± 3.90)**	

Table 5: A Comparison of Hours in Co-Culture Cloned Embryos to Reach the Stages of 8 Cells, Morulae and Blastocysts between the Across Species of Somatic Ear Cells of Cattle (Se of Ct) Fused Into Swamp Buffaloes Oocytes (Sb Oocytes) and Somatic Ear Cells of Swamp Buffaloes (Se of Sb) Fused into Cattle Oocytes (Ct Oocytes).

bull already showing her fast puberty as a beef breed (see pictures 1 and 2).

Cloning of buffalo was only working progressively in the laboratory level due to the difficulty to prepare buffalo recipients. Swamp buffaloes, belonging to the small farmers, are far away from the laboratory and they are not normally inseminated. Swamp buffaloes show very weak oestrous symptom insult in irregular date detection to transfer the cloning embryos. It is necessary to nurture the buffaloes and to educate the owners: how to rearing their animals in intensive program liked dairy cattle or intensive breeding beef cattle. It is, therefore, that such farms and animals would be ready to prepare as the recipients for receiving cloning embryos from the laboratory.

During years 2001-2003, Professor Maneewan Kamonpatana, the author present her attitude and willingness to share the scientific mind with the public. Some of her key points must be mentioned here.

The main reason of "Cloning: why in cows" may be focusing on her willingness and wish to serve the advanced technologies under the direction to the ordinary farming production. She used to mention that it was not worth for a University to set up the farm just only for the research. Her talent and energetic work during her 30 years experience, to establish the cooperation among farmers to utilize her given technologies, were proven to be the most economical way and were directed to production scale without delay or misleading through the step of so called "*Technology transfer*". She used to guide her staffs to work directly at the farms in the manner of integrated five technologies all together as follow:

1. Early pregnancy diagnosed by progesterone test;
2. Reproductive management using semi-manual computer;
3. Mato-Matid Technology for increasing production;
4. CMN block for enhancing digestively in rumen (C for Carbohydrate, M for Minerals and N for Non-protein nitrogen);
5. Artificial breeding of
6. a) test tube embryos,

b) embryos transfer and

c) cloning

It was not common for Thailand in the past decade to generate the success of cloning in livestock such as cows. The public must look carefully how such an event occurred by her team. She and her team have been created a formula of setting up the functional group to contribute both education and livestock production with advanced technologies in Thai scientists' hand.

Her success with the establishment of both laboratory and farming services must be used as a role model for the others who involved in genetic and molecular science of biological production.

Intelligence of utilization natural genetic pools of cattle and buffaloes is also one of her direction to persuade the cloning existence. Domesticated buffaloes and cattle are the rich genetic pools in farmers' hand. The achievement of *Ing* and *Nikolaus* are the evidences to support this utilization of farmers' genetic pool for producing reconstructed cloning embryos.

Since 1953 Francis Crick and James Watson discovered the structure of DNA, scientists around the world did work hard to find out the techniques of artificial breeding and in conjunction to gene manipulation. Experimental animals and farm animals have been widely used in this area. Recently somatic cells, as the donors of genetic selection, have been proven to be successful in producing the cloning embryos. *Ing* and *Nikolaus* are the cases to demonstrate that somatic ear cells are genetic donors to fuse with ooplasm of the non identified oocytes to reconstruct the cloned embryos. The recipients of the same species could accept the cloned embryos and were pregnant to full term and giving birth the calves safely.

However percentage of success is low. It is remained to investigate further. It is quite important to point out that well preparation of recipients in the tropical zone is one of most key success in having *Ing* and *Nikolaus*. Professor Maneewan Kamonpatana has invented the immunogens so called "Mato-Matid" in which the agent "Matid" does help the recipients to generate multi-corpus lutea for supporting the pregnancy. And the agent "Mato" does enhance the body performance of the animals to have early puberty, healthy and fertility.

Fifty years since Crick and Watson found the structure of DNA. Thailand and Thai scientists have been capable to catch up with the World class scientific invention. It is an elegant event of science and

technology that encourage the Thai educators and scientists to work harder in order to get along with the world scientists to utilize the natural genetic pools wisely. Everyone should realize that the most intelligence of genetic pools is in the nature. Natural resource is the main library for mankind. Scientists who work on gene manipulation must respect to the great gene library on which nature control, manage and situate in each habitat systematically [20].

So far, artificial breeding in livestock is not yet reach the level of commercialization. Scientists must work hard and get along with the nature. What is life: A major question remained to be learn and a great deal of mechanism is still unknown.

Thailand is a country that is very rich in habitats. Thai scientists should concentrate their minds to learn what the nature has given to people who live in this land at the nano level. Nanotechnology of food chain beside the protein from beef will be the excellent target for Thai scientists to work together in harmony.

Thai scientists have an excellent opportunity to investigate the *food chain* at nano level. Thailand as a food producing country, it may be worth-while to popularize the concept of food production in the manner of *food chain*.

Food chain means consequent food productivity from one species to the other. It would be an intellectual invention to setting up a habitat that allows man, animals, plant and microorganism can live together with food chain in the habitat. The nutrients in food would be moving from one species to the other with a rotated system. People could able to have both food safety and environment protection at the same time. This concept should be demonstrated and popularized at worldwide level.

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