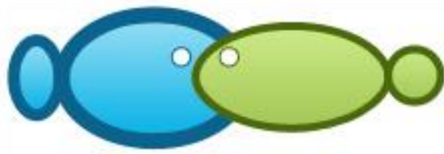

Motility and fertility rates of cryopreserved and freshly collected milt of FAC red Nile tilapia (*Oreochromis* sp.) and determination of the hatching rate via *in vitro* fertilization

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Abstract. This study evaluated the differences between cryopreserved (treatment 1) and freshly collected (treatment 2) milt of FAC red tilapia by *in vitro* fertilization. The study had three sets of tests: i) motility scoring, ii) milt viability testing in respect to fertility rate at 2.5×10^8 mL^{-1} milt concentration, and iii) hatching rate using the treatments. Three groups (G1, G2 and G3) representing the sets of experiments used one female and five males of broodstock. Using freshly collected milt, the motility rate of each group had the same score of 10. After cryopreservation, the rate decreased and ranged to 5-6. In terms of fertility rate, no significant differences (p>0.05) were observed between fresh and cryopreserved milt in all groups. Among the three groups, G1 hatching rate from eggs fertilized with cryopreserved milt (19.48±4.52) was significantly lower (p>0.05) than the hatching rate obtained using fresh milt (52.2±1.63). No significant differences (p>0.05) were observed among the treatments in G2 and G3. This study found that cryopreservation lowers the motility rate of milt, but presents no differences to fresh milt in terms of fertilization rate and hatchability.
Key Words: breeding, broodstock culture, cryopreservation.

Introduction. Tilapia aquaculture is rapidly expanding, with the national production of the Philippines reaching 307878.28 metric tons during 2023 (PSA 2024). The production is estimated to increase by 2030 (Arumugam et al 2023). One of the factors that causes this rapid increase is the introduction of improved strains of Nile tilapia (*Oreochromis niloticus*), which is the major farmed tilapia species (Teoh et al 2011).

One of the major breakthroughs of the Freshwater Aquaculture Center, Central Luzon State University, Philippines, is breeding the faster-growing red Nile tilapia. According to Fang et al (2022), red tilapia is largely demanded by consumers because of its color associated with some premium reef and marine fishes of similar coloration, like the red sea bream (*Pagrus major*), red snapper (*Lutjanus campechanus*) and grouper (*Epinephelus chlorostigma*). Red tilapia is a cheaper alternative to the said high-priced marine species, explaining the increasing acceptance from consumers; hence, the fry production and demand may increase (www.pcaarrd.dost.gov.ph).

Hagedorn et al (2018) suggested that cryopreservation can provide a year round supply of desired species, regardless of the spawning season. It can allow hybridization, has greater care in carrying out selective breeding and can improve stocks. Cryopreservation includes freezing, storing, and thawing of cells, tissues, or whole organism, so that the result is viable, resembles the starting material (Devita et al 2021), with essentially no detectable biochemical activity and can be preserved for years, and possibly for centuries (Chian 2010). One way to evaluate the efficiency of the preserved germplasm is by applying it to the subject species using asexual fertilization.

In vitro fertilization (IVF) is a procedure in which milt is placed in a special dish with unfertilized eggs to achieve fertilization and can be transferred into the uterus or

frozen (cryopreserved) for future use (Żarski et al 2014). Researches showed that, through IVF, there will be rapid expansion of the animal population as a result of the large number of zygotes that can be made and because of controlled environmental conditions (Herrick 2019).

This study on cryopreservation of red tilapia determined the motility, fertility, and hatching rate differences between the preserved and freshly collected milt. Studies that compare the effectivity of cryopreservation and freshly collected milt are seldom, specifically with regards to the capability to hatch eggs.

Material and Method

Broodstock origin and management. Red tilapia bred at the Freshwater Aquaculture Center - Central Luzon State University were used as experimental fish. Male and female broodstock were kept separately in different tanks (tank 1 and tank 2). Broodstock groups were fin clipped for ease of identification and fed twice daily on a commercial diet of approximately 4% protein.

Experimental treatment. There were three groups (sets of experiment), comprised of 1 female and five males of broodstock used in each group (G1, G2, G3). Different female broods were used in every group and all five male broods were used in all groups. The milt collected was pooled to ensure the milt concentration used in the study. Freshly collected milt served as treatment I, while treatment II was represented by the collected milt subjected to cryopreservation. Each treatment for each group was replicated three times, thus dividing the collected milt and eggs into three.

Collection of eggs. Females were checked for signs of readiness to spawn. The indicator for readiness to spawn is characterized by pinkish to reddish color of the genital papilla, fully opened and protruding, and slightly distended abdomen. The females were removed from the tank and eggs were stripped manually. Stripping was done by applying a gentle downward pressure with the thumb across the abdomen in an anterior-posterior direction. The collected eggs were transferred in a Petri dish and covered until use.

Collection of milt. A good sample of milt is free from water, urine and fecal contamination. To minimize milt contamination, the bladder of each male was manually stripped of urine and the genital area was dried using a towel. Microcentrifuge tubes were used to collect milt. A 100 µL capillary tube was positioned at the tip of the genital papilla and the microcentrifuge tube was placed at the other end for the purpose of collecting milt. By applying a gentle pressure on the abdomen, the milt was drawn into the tube by capillary attraction. The collected milt was checked by placing a drop of milt from each capillary tube on a glass slide for microscopic examination, to ensure the presence of the immotile state. The motility of the milt was also checked by adding a few drops of water on the glass slide. Only samples with a 9-10 motility score (Table 1) after activation were used. Pooled milt from different males was used for cryopreservation and *in vitro* fertilization. The milt was transferred into a labelled microcentrifuge tube and stored in a refrigerator at 4°C until use.

Estimation of milt density. Milt concentration was estimated using a Neubauer counter (Haemacytometer, 0.1 mm, 1/400 mm^2, Weber Scientific, England). Before the milt was used, spermatozoon count was estimated from the whole milt sample density. Dilution was done by taking 10 µL of milt sample added in 490 µL of diluent (Modified Fish Ringer), making 500 µL of milt suspension in a microcap vial. From the first dilution, 10 µL was drawn and added in 90 µL of diluent making a 1/50 and 1/10 dilution, respectively. A small amount of sample was dropped on the haemacytometer for counting. Concentration of milt per mL was calculated using the formula:

Concentration= Total number of cells from five diagonal squares x dilution factor x 50000

A dilution factor concentration of 2.5×10^8 mL^{-1} was used for cryopreservation and fertilization.

Motility scoring. The milt motility in all samples was scored on a rating scale system of 0 to 10 (Table 1). Scoring was done by diluting 20 μL of sample in 100 μL of water. Scoring was done for the whole sample, being divided after observation for replication. The equivalent for the observation for each sample to the motility score was based on the percentage of motile spermatozoa to the total number of spermatozoa per sample.

Table 1

Motility scoring

Criteria	*Motility score*
100% of the spermatozoa are motile	10
90% of spermatozoa are motile; all other spermatozoa exhibiting strong vibrations of the loco	9
80% of spermatozoa are motile; all others with vibrating loco	8
70% of spermatozoa are vibrating; all others exhibit motion	7
60% of spermatozoa are vibrating in loco; a few spermatozoa motile	6
50% of spermatozoa are immotile; a few slightly vibrating and in progressive motion	5
30-40% of spermatozoa are immotile with very few slightly vibrating; occasionally one spermatozoid could be observed in progressive motion	4-3
10-20% of spermatozoa are immotile; occasionally, some are slightly moving	2-1
Spermatozoa not moving	0

Table 2 shows the chemical composition of the Fish Ringer Solution. This solution was modified and used as diluent in all the experiments. The modified solution had a pH of 8. Milt samples were stored at 4°C until use.

Table 2

Chemical composition of the modified Fish Ringer Solution

Chemical composition	*Amount*
Sodium chloride (NaCl)	3.25 g
Potassium chloride (KCl)	1.50 g
Sodium bicarbonate ($NaHCO_3$)	0.10 g
Calcium chloride ($CaCl_2$)	0.15 g
Distilled water	500 mL

Cryopreservation. The cryopreservation in the study was conducted after the desired concentration of the milt was achieved. Milt with concentrations of 2.5×10^8 mL^{-1} was cryopreserved using a dilution rate of 9:1 (milt suspension:methanol) (Rana & McAndrew 1989; Danting 1992). The cryoprotectant used was methanol. During the equilibration period of 30 min, the milt suspensions were drawn into plastic straws with a capacity of 250 μL. Before loading the samples in a programmable cooling chamber, pre-freezing scoring of milt motility was carried out. Using a programmable cooling/thawing chamber, the samples were cooled at 5°C down to -60°C by decreasing the temperature (-5°C every 2 min). The straws were plugged and stored in liquid nitrogen for 1 week.

In vitro fertilization. For milt viability testing, fertilization of eggs involves the addition of 400 μL of water during fertilization. The stripped eggs were divided into several aliquots (80-150 eggs each). Each egg aliquot was fertilized by adding milt with cell concentrations of 2.5×10^8 mL^{-1}. Likewise, the cryopreserved milt was thawed in a water bath at 40°C for 8 seconds using a thawing machine. Both ends of the straws were cut and the milt suspensions were emptied over the egg aliquots. The milt suspension used

for treatment I (freshly collected milt) was stripped from the same males from which the milt had been cryopreserved beforehand. After the addition of water, the eggs were gently shaken for 45 seconds to activate the milt and then were let to stand for 5 min. The eggs were rinsed several times with clean water from the incubator and were transferred to incubator jars (Rana & McAndrew 1989; Danting 1992). The water flow kept the eggs turning, thus mimicking the movement of the eggs in the buccal cavity of the female fish. Morula scoring was done 4-6 h after fertilization using a microscope. The numbers of viable, non-viable pigmented and unpigmented eggs were noted. Scoring of hatching rate was done 5-6 days after fertilization. The hatched and dead larvae were also recorded. This procedure was followed for the *in vitro* fertilization of the eggs. The water parameters were similarly maintained for the two treatments as a recirculating system was utilized on both treatments.

Determination of the fertility rate was based on Liu et al (2012), while hatching rate computation was based on Akpoilih & Adebayo (2010). Fertility and hatching rates were determined as follows:

Fertility rate (%) = (Total number of embryo in morula stage)/(Total number of eggs) × 100

Hatching rate (%) = (Total number of hatchlings)/(Total number of fertilized eggs) × 100

Statistical analysis*.* Data analysis was performed using SPSS (Statistical Package for Social Sciences) version 16.0. The differences of means between measured parameters were determined using the T-test.

Results. Table 3 presents the means of motility scores of freshly collected and cryopreserved milt from the two treatments of the three groups.

Table 3

Motility scores of freshly collected and cryopreserved milt from the two treatments of the three groups

Group	*Treatment*	
	I (fresh milt)	*II (cryopreserved milt)*
1	10	6
2	10	5
3	10	5

Note: data are means of three replicates.

The motility rate in treatment I had a mean of 10 in all groups. For treatment II, group 1 had a rate of 6, while both groups 2 and 3 had a motility score of 5. Scoring was done for the whole sample, it was then divided after observation for replication, thus standard deviations of all groups in all treatments were 0, and t could not be computed.

Table 4 represents the data for freshly collected and cryopreserved milt's fertility rate. Table 5 presents the summary data on the total number of hatched larva produced from freshly collected and cryopreserved milt over the fertilized eggs.

For hatching rate, the data were also analyzed separately according to designated group. For group 1, TI had a mean of 52.2±1.63% and TII had 19.48±4.52%. For group II, TI had a mean of 5.95±5.84%, while TII had 6.87±4.35%. Group 3 TI had a mean of 30.88±8.2% and TII had 22.43±10.51%. Table 5 shows that there was a significant difference ($p<0.05$) between TI and TII in group 1. The T-test also showed that no significant differences ($p>0.05$) were observed among the treatments in groups 2 and 3.

Table 4

Fertility rate (%) of freshly collected and cryopreserved milt

Group	*Treatment*	
	I (fresh milt)	*II (cryopreserved milt)*
1	88.28±7.26[a]	89.10±1.20[a]
2	82.91±3.17[a]	83.13±2.24[a]
3	96.62±0.8[a]	96.9±0.2[a]

Note: data are means of three replicates±SD; means in the same row with the same superscript had no significant differences (p>0.05).

Table 5

Summary data on hatching rate (%) using freshly collected and cryopreserved milt

Group	Treatment	
	I (fresh milt)	*II (cryopreserved milt)*
1	52.2±1.63[a]	19.48±4.52[b]
2	5.95±5.84[a]	6.87±4.35[a]
3	30.88±8.2[a]	22.43±10.51[a]

Note: data are means of three replicates±SD; mean in a row with the same superscript letters are not significantly different at (p>0.05).

Discussion. In the study conducted by Viveiros et al (2010) that compares a computer-assisted milt analyzer (CASA) system versus subjective microscopic examination in assessing milt motility, there was no difference (p>0.05) between subjective or CASA assessment of post-thaw milt motility. Thus, it was concluded that although the CASA system provided objective data regarding milt motility, subjective evaluation of milt motility was practical and a good indication of milt quality and could readily be done by well-trained personnel under field or laboratory conditions.

In the present study, all the motility rate of the groups decreased as the milt was cryopreserved. Such effect was also observed by Danting (1992). From the study conducted by Viveiros et al (2012), the subjectively assessed *Brycon insignis* sperm motility decreased from 100% motile sperm and a quality score of 5 in fresh sperm to 54% motility and a quality score of 3 in postthaw sperm. The same observation was reported by Nynca et al (2014) that in cryopreservation of brown trout (*Salmo trutta* m. *fario* L.) semen, the proportion of motile cells decreased faster with time in thawed milt samples than in fresh ones and movement duration was also affected. Kommisrud et al (2020) reported that cryopreserved milt of Atlantic salmon (*Salmo salar* L.) was immotile, and that the motile cells rapidly lost their motility and swimming velocity after dilution. According to the study of Ciereszko et al (1999), cryopreserved milt of rainbow trout (*Oncorhynchus mykiss*) had 0% motility; however, when used, it resulted in 67.9% fertilization success. These findings coincide with the findings of Danting (1992), thus providing evidence that there is no conclusive relationship between motility and fertility.

The fertilization ability of cryopreserved milt did not differ significantly from that of fresh milt. The same results were observed from the study conducted on cryopreserved milt of common carp (*Cyprinus carpio*) (Magyary et al 1996), African catfish (*Clarias gariepinus)* (Omitogun et al 2010), rainbow trout (Ciereszko et al 2014), red snapper (*Lutjanus argentimaculatus*) (Vuthiphandchai et al 2009), spotted halibut (*Verasper variegatus*) (Tian et al 2008) and turbot (*Psetta maxima*) (Suquet et al 1998) compared to freshly collected milt. Possible explanations for this phenomenon of cryopreserved milt was suggested by Muchlisin (2005), with motility activated by an egg factor, as eggs can be fertilized by immotile cells (Danting 1992). Muchlisin (2005) also proposed the existence of a factor in egg and ovarian fluid that can stimulate motility and could fertilize the egg, supporting the theory that milt motility may be activated by an

egg factor. Muchlisin (2005) noted that the mechanism for the stimulation of milt motility by the egg factor remains unclear. Muchlisin (2005) suspected that some of the steroid hormones in eggs and ovaries may play a role in this phenomenon.

Lujić et al (2015) added that extenders and cryoprotectants in different concentrations are able to preserve cell integrity and cellular functions of milt during cryopreservation (that may cause cryoinjury to the cell), favorable osmotic conditions of the extender and cryoprotectant being needed for fertilization. In the present study, methanol was used as cryoprotectant. Results from the three groups can support the observation of Kommisrud et al (2020) that fertilization can be considered the most effective method to evaluate the quality of thawed milt.

Different studies have demonstrated varying hatching rates between cryopreserved and freshly collected milt. The studies conducted on African catfish (Oteme et al 1996), common carp (Linhart et al 2000), silver barb (*Barbodes gonionotus*) (Vuthiphandchai et al 2015), and turbot (Suquet et al 1998) reported insignificant differences between hatching rates from eggs fertilized with fresh and cryopreserved milt.

Among the three groups of red tilapia, G1 hatching rate from the treatment with cryopreserved milt was significantly lower than the hatching rate using fresh milt. The same results were observed for long tooth grouper (*Epinephelus bruneus*) (Oh et al 2013) and paddle fish (*Polyodon spathula*) (Mims & Shelton 2015). According to Oh et al (2013), acrosomal damage could have been a major factor in the low hatching rate and increasing the post-thaw milt -to-egg ratio could partially compensate for the low viability of cryopreseved milt. Oh et al (2013) suggested that acrosomal damage probably occurred either during freezing or thawing and probably resulted in a premature acrosomal reaction, observations made by electron microscopy. Hence, poor thawing procedure can be the cause of poor viability of cryopreserved milt in group 1, while groups 2 and 3 may have had a better response to the thawing procedure.

Conclusions. This study concludes that cryopreservation of FAC red tilapia milt lowers the motility rate of the milt, but there were no differences between cryopreserved and fresh milt in the fertilization of eggs and the ability to hatch. A direction of study recommended is to have more series of experiments or trials to have a better comparison between cryopreserved and fresh milt. Future directions can also focus on comparisons of motility scoring between subjective observations and the use of machines, damage determination for milt morphology and function after freezing and thawing, and the determinations of other parameters (like milt density, plasma composition, head morphology or ATP concentration) that may better characterize the effectivity of cryopreservation.

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Conflict of Interest. The authors declare that there is no conflict of interest.

References

Akpoilih B. U., Adebayo O. T., 2010 Effect of formalin on the hatching rate of eggs and survival of larvae of the African catfish (*Clarias gariepinus*). Journal of Applied Sciences and Environmental Management 14(4):31-34.

Arumugam M., Jayaraman S., Sridhar A., Venkatasamy V., Brown P. B., Kari Z. A., Tellez-Isaias G., Ramasamy T., 2023 Recent advances in tilapia production for sustainable developments in Indian aquaculture and its economic benefits. Fishes 8(4):176.

Chian R. C., 2010 Cryobiology: an overview. In: Fertility cryopreservation. Chian R. C., Quinn P. (eds), Cambridge University Press, pp. 1-9.

Ciereszko A., Dabarowski K., Lin F., Christ S. A., Toth G. P., 1999 Effect of extenders and time of storage before freezing on motility and fertilization of cryopreserved muskellunge spermatozoa. Transaction of the American Fisheries Society 128:542-548.

Ciereszko A., Dietrich G. J., Nynca J., Dobosz S., Zalewski T., 2014 Cryopreservation of rainbow trout semen using a glucose-methanol extender. Aquaculture 420-421:275-281.

Danting M. J., 1992 Optimization of UV irradiation treatment and cryopreservation of *O. niloticus* spermatozoa for induced gynogenesis. MS Thesis, University of Stirling, Scotland, 74 p.

Devita A., Abinawanto, Lestari R., 2021 The effect of coconut milk in various concentrations on spermatozoa motility of koi fish (*Cyprinus carpio*, Linnaeus 1758) 48 hours post-cryopreservation. Journal of Physics: Conference Series 1725:012061.

Fang W., Huang J., Li S., Lu J., 2022 Identification of pigment genes (melanin, carotenoid and pteridine) associated with skin color variant in red tilapia using transcriptome analysis. Aquaculture 547:737429.

Hagedorn M. M., Daly J. P., Carter V. L., Cole K. S., Jaafar Z., Lager C. V., Parenti L. R., 2018 Cryopreservation of fish spermatogonial cells: the future of natural history collections. Scientific Reports 8:6149.

Herrick J. R., 2019 Assisted reproductive technologies for endangered species conservation: developing sophisticated protocols with limited access to animals with unique reproductive mechanisms. Biology of Reproduction 100(5):1158-1170.

Kommisrud E., Myromslien F. D., Stenseth E. B., Zeremichael T. T., Hofman N., Grevle I., Sunde J., 2020 Viability, motility, ATP content and fertilizing potential of sperm from Atlantic salmon (*Salmo salar* L.) in milt stored before cryopreservation. Theriogenology 151:58-65.

Linhart O., Rodina M., Cosson J., 2000 Cryopreservation of sperm in common carp *Cyprinus carpio*: sperm motility and hatching success of embryos. Cryobiology, 41(3):241-250.

Liu Q. H., Ma D. Y., Li J., Xu S. H., Xiao Y. S., Xiao Z. Z., 2012 Marine fish sperm cryopreservation and quality evaluation in sperm structure and function. In: Current frontiers in cryopreservation. InTech.

Lujić J., Bernáth G., Marinović Z., Radojković N., Simić V., Ćirković M., Urbányi B., Horváth A., 2015 Fertilizing capacity and motility of tench *Tinca tinca* (L., 1758) sperm following cryopreservation. Aquaculture Research 48(1):102-110.

Magyary I., Urbanyi B., Horvath L., 1996 Cryopreservation of common carp (*Cyprinus carpio* L.) sperm II. Optimal conditions for fertilization. Journal of Applied Ichthyology 12(2):117-119.

Mims S. D., Shelton W. L., 2015 Paddlefish aquaculture. John Wiley & Sons, 299 p.

Muchlisin Z. A., 2005 Current status of extenders and cryoprotectants on fish spermatozoa cryopreservation. Biodiversitas 6(1):66-69.

Nynca J., Dietrich G. J., Dobosz S., Grudniewska J., Ciereszko A., 2014 Effect of cryopreservation on sperm motility parameters and fertilizing ability of brown trout semen. Aquaculture 433:62-65.

Oh S. R., Lee C. H., Kang H. C., Song Y. B., Kim H. B., Lee Y. D., 2013 Evaluation of fertilizing ability using frozen thawed sperm in the longtooth grouper, *Epinephelus bruneus*. Development & Reproduction 17(4):345-351.

Omitogun O. G., Olaniyan O. F., Oyeleye O. O., Ojiokpota C., Aladele S. E., Odofin W. T., 2010 Potentials of short term and long term cryopreserved sperm of the African giant catfish (*Clarias gariepinus* Burchell, 1822) for aquaculture. African Journal of Biotechnology 9(41):6973-6982.

Oteme Z. J., Rodriguez J. N., Kouassi C. K., Hem S., Agnese J. F., 1996 Testicular structure, spermatogenesis and sperm cryopreservation in the African clariid catfish *Heterobranchus longifilis*. Aquaculture Research 27(11):805-813.

Rana K. J., McAndrew B. J., 1989 The viability of cryopreserved tilapia spermatozoa. Aquaculture 76(3-4):335-435.

Suquet M., Dreanno C., Petton B., Normant Y., Omnes M. H., Billard R., 1998 Long-term effects of the cryopreservation of turbot (*Psetta maxima*) spermatozoa. Aquatic Living Resources 11(1):45-48.
Teoh C. Y., Turchini G. M., Ng W. K., 2011 Genetically improved farmed Nile tilapia and red hybrid tilapia showed differences in fatty acid metabolism when fed diets with added fish oil or a vegetable oil blend. Aquaculture 312(1-4):126-136.
Tian Y. S., Chen S. L., Ji X. S., Zhai J. M., Sun L. J., Chen C., Su P. Z., 2008 Cryopreservation of spotted halibut (*Verasper variegatus*) sperm. Aquaculture 284(1-4):268-271.
Viveiros A. T., Isaú Z. A., Caneppele D., Leal M. C., 2012 Sperm cryopreservation affects postthaw motility, but not embryogenesis or larval growth in the Brazilian fish *Brycon insignis* (Characiformes). Theriogenology 78(4):803-810.
Viveiros A. T., Nascimento A. F., Orfão L. H., Isaú Z. A., 2010 Motility and fertility of the subtropical freshwater fish streaked prochilod (*Prochilodus lineatus*) sperm cryopreserved in powdered coconut water. Theriogenology 74(4):551-556.
Vuthiphandchai V., Chomphuthawach S., Nimrat S., 2009 Cryopreservation of red snapper (*Lutjanus argentimaculatus*) sperm: effect of cryoprotectants and cooling rates on sperm motility, sperm viability, and fertilization capacity. Theriogenology 72(1):129-138.
Vuthiphandchai V., Wilairattanadilok K., Chomphuthawach S., Sooksawat T., Nimrat S., 2015 Sperm cryopreservation of silver barb (*Barbodes gonionotus*): cryoprotectants, cooling rate and storage time on sperm quality. Aquaculture Research 46(10):2443-2451.
Żarski D., Horváth A., Bernáth G., Palińska-Żarska K., Krejszeff S., Müller T., Kucharczyk D., 2014 Application of different activating solutions to in vitro fertilization of crucian carp, *Carassius carassius* (L.), eggs. Aquaculture International 22:173-184.
*** PSA, 2024 Fisheries situation report, January to December 2023. Available at: https://psa.gov.ph
*** www.pcaarrd.dost.gov.ph

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