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Pituitary Extraction: An Evaluation of Its Gonadotropic Activity Via Semen Characterization of African Catfishs Milt Diluted And Stored In Different Extenders

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Abstract
This study was designed to evaluate pituitary function and its gonadotropic activity by inference via characterization of semen (milt) parameters of African catfish following extension in different milt extenders including Soya milk, Coconut water and Coconut milk based extenders to justify pituitary extraction as well as compare longevity of the germ cells in those media and see possibility of replacing egg yolk based extenders with those media for Catfish milt under chilled condition. Pooled milt from 5 male brood stocks of catfish aged 16-18 months old and weighing 1.25-1.60kg and measuring 52.4-54.0cm in length were used for this experiment. Volume of semen was observed and recorded. The color was graded from creamy to watery. A drop of fresh semen was made on a pre-warm glass slide and covered with a warm cover slip. The sample was viewed under light microscope starting from x4 magnification to observe wave motion and then individual motility at x10 magnification. The extenders used for this experiment included Citrated egg yolk, as control. Phytochemical analysis was carried out to know the constituents present in the diluents. Data were analyzed using ANOVA. From the result of the study we found out that gonadotropic activity and pituitary function were evidenced by the relatively long term survival (7 days) of the milt samples in the favorable extenders. Results also showed that citrated egg yolk and the mixture (soya milk and coconut water) were able to maintain semen viability more than other extenders. Thus, soya milk and the mixture of soya milk + coconut water proved to be more potent semen extenders for the African catfish milt It was concluded that by inference pituitary extraction of the African catfish could substitute endogeneous and exogeneous gonadotropins in estrous synchronization protocols in mammals including domestic ruminants. Citrated egg yolk and the mixture were most favourable semen extenders for the catfish. Hence, it was recommended that further studies be done in the application of the gonadotropin of pituitary extract origin in synchronization protocols in domestic animals.

Keyword: *Catfish, Pituitary, Gonadotropic, Milt, Extender, Longevity*

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I. Introduction

The deposition manually of spermatocytes into a receptive female is referred to as Artificial insemination (Foote, 1968). Artificial insemination (A.I.) as practiced by bees and many other flying insects has played an important role in plant reproduction for a very long time. In animal production, undocumented tales exist of Arabs obtaining sperm from mated mares belonging to rival groups and using the sperm to inseminate their own mares. Pioneering efforts to establish A.I. as a practical procedure were begun in Russia (Ivanow 1899). Ivanow (1907) studied A.I. in domestic farm animals, dogs, foxes, rabbits and poultry. Leeuwenhoek and his assistant, Hamm, were the first persons to see sperm cell which they called "animalcules." Leeuwenhoek did not have an advanced formal education, so he did not study Latin, the scientific language of the day. However, he was a clever, capable individual who ground lenses so precisely that sperm were visible.

The most widely used technique for artificial breeding of cattle and buffalo requires a perfect medium to extend and preserve the semen ejaculate from elite bulls to maximize usage of the superior quality of male germplasm. Semen extenders possess properties that protract the longevity of spermatozoa in extended form during harsh ambient conditions and cryopreservation (Feizollahzadeh *et al.,* 2017). Spermatozoa during storage are affected by osmotic changes, pH fluctuations, energy depletion during metabolism, cold shock and cryo-damages during freeze-thawing procedures (Ibrar Muhammad Khan, Z.C, 2021 2021). During cryopreservation, cholesterol to phospholipid ratio of sperm bio-membranes gets disturbed mainly due to cholesterol efflux and generation of numerous reactive oxygen species (ROS). All these disturbances directly compromise

spermatozoa fertility. Therefore, a combination of good quality semen extender and additives must be used in such a way that fertility of spermatozoa can be retained outstandingly during semen preservation

According to Aboagla and Terada 2004, egg yolk can be generally accepted to be an effective agent in semen extenders for protection of spermatozoa against cold shock and lipid-phase transition effect. However, the use of chilled-stored semen diluted in egg yolk-based semen extenders is limited by its relatively short-time fertilization capacity (Aurich *et al.*,1997).The potential cause of decline in motility and fertility during hypothermic storage of liquid semen is an oxidative damage of spermatozoa (Wiktoria Kurkowska, 2020).Hence, to maintain sperm for longer period cryopreserved and cooled, it is important to dilute semen in a protective solution (AX, 2000).

The addition of sodium citrate in yolk phosphate buffer has increased the survival of sperm cell up to three to four days at 5^0C and increased the sperm visibility by dissolving the fat globules (Nagasa, 1954). Prior to this, Philips and Lardy (1940) have documented that yolk phosphate buffered extender was first reported as the first semen extender in United State of America. Semen extenders can be produced from various sources includingQuail egg yolk. Soya milk, Coconut water and Coconut milk.

Nutritional composition research has shown that eating a well-balanced food can improve human health. Variety of food, including vegetables, fruits, grain, and protein, is essential to get the full range of nutrients for good health. The right balance of calories, protein, fat, carbohydrates, vitamins, and minerals provides energy, and the variety of nutrients growing children and working adult need. Both Child and Adult Care Program (CACP) meal pattern and the Pyramid website by United State of America, Department of Agriculture, Food and Nutrition Service, encourage eating a variety of foods (USDAFN, 2005). The most important health benefits of quail eggs include their ability to improve vision, boost energy level and stimulate growth and repair of tissue (Beautymunsta,2007). Quail eggs also help to improve metabolism, reduce blood pressure, soothe allergies, cleanse the body, and prevent chronic diseases.

Studies reported that quail eggs are packed with vitamins and minerals and their nutritional value is three to four times greater than chicken eggs(Lalwani 2011) Regular consumption of quail eggs helps fight against many diseases which is a natural combatant against digestive tract disorders such as stomach ulcers (Lee *JM,* 2000). Quail eggs strengthen the immune system, promote memory health, increase brain activity and stabilize the nervous system (Lalwani ,2011). They are useful in the management of anemia by increasing the level of hemoglobin in the body while removing toxins and heavy metals (Thomas D Coates, 2014). Chinese use quail eggs to help treat tuberculosis, asthma, and even diabetes. Quail eggs can help prevent sufferer of kidney or gallbladder stones and remove these types of stones. The nutritional value of quail eggs is much higher than those offered by other eggs and they are rich sources of antioxidants, minerals, and vitamins(Lalwani, 2011).

Quail eggs contain 13 percent proteins compared to 11 percent in chicken eggs (Milner et al.,2005). Quail eggs also contain 140% vitamin B1 compared to 50% in chicken eggs. In addition, quail eggs provide five times as much iron and potassium(Bjelacovic, 2007).

Soya milk is a natural product derived from soya beans. It is one of the most popular milk-substitute for individuals with lactose-intolerance or those with allergy to cow milk (*Sethi,* 2016). Soya milk is one of the most important products from soya beans because of its hypolipidemic effect. This is mainly due to the fact that they comprise a variety of nutrients, which have shown to exert a potential role in lipid metabolism including quality protein, polyunsaturated fatty acids, saponins, phytostrols, soya lecithins and isoflavones .

Coconut water is made of 95% water but at the same time, it offers a unique chemical composition that features vitamins, minerals, amino acid, natural sugar and phytohormones. Its useful components are cytokinins, a class of phytohormones that boast anti-aging, anti-thrombotic and anti-carcinogenic effect

Coconut milk is distinguished from coconut water by its thicker consistency and milkier appearance. Unlike coconut water, which is the liquid found directly inside a coconut (Philippine, 2016). Coconut milk contains 230 kilocalories and is 68% water, 24% total fat, 6% carbohydrates, and 2% protein. The fat composition includes 21 grams of saturated fat, half of which is lauric acid (USDA,2016). One of the prominent components of coconut milk is coconut oil, which health organizations such as United States Food and Drug Administration, World Health Organization discourage people from consuming in significant amounts due to its high levels of saturated fat. Excessive coconut milk consumption can also raise blood levels of cholesterol due to the amount of lauric acid, a saturated fat that contributes to higher blood cholesterol by increasing the levels of low density lipoprotein cholesterol (Mensik, 2003; Eyres, 2016)

Aim

The aim of this work was to evaluate pituitary function and its gonadotropic activity by inference via characterization of semen (milt) parameters of African catfish following extension in different milt extenders including Soya milk, Coconut water and Coconut milk based extenders to justify pituitary extraction as well as

compare longevity of the germ cells in those media and see possibility of replacing egg yolk based extenders with those media for Catfish milt under chilled condition.

II. Materials and Method

2.1: Experimental Design

Five semen extenders were prepared to form 5 groups designated A, B, C, D and E. Group A represent Citrated egg yolk which served as control, while groups B, C, D and E represent Soya Milk, Coconut Milk, Coconut water, and Mixture (Soya Milk and Coconut Water) respectively.

2.2: Preparation of Buffer and Semen Extendes

The preparation of 2.9% tri-sodium citrate solution was made by dissolving 2.9g of the salt in 20ml of distilled water in flat bottom flask. This was shaken together until the salt dissolved completely. The solution was then made up to 100ml by adding distilled water.

2.2.1: Preparation of Egg Yolk

Freshly laid eggs were collected from quail and poultry birds. These eggs were washed and disinfected using 70% alcohol. They were cracked carefully into two, such that the albumen drained of from the crack until little of it was left with the yolk. The yolk was then carefully dropped on Whatman filter paper which absorbed what was left of the albumen. The yolks were collected into beakers.

2.2.2: Preparation of Citrated Egg Yolk

80ml of citrated water was made up to 100ml with 20ml of quail egg yolk. 0.6ml penicillin/streptomycin injection was constituted (100,000 i/u and 200mg) added to the solution. The mixture was thoroughly mixed. 10ml of the preparation was collected into a tube and maintained in warm water at 37^0C for onward extension.

2.2.3: Preparation of Soya Milk

Soya beans were soaked in clean water for 10-12 hours. The shafts were removed using a clean sieve. The harvested soya beans were ground, sieved and the filtrate was separated from the sediments. The filtrate was boiled until it changed from slight creamy to milky color. It was allowed to cool at room temperature and then decanted into sterile container.

2.2.4: Preparation of Coconut Milk

The coconut was removed from the shell and then grated. About 20 ml of distilled water was added to the grated coconut. The solution was then sieved and the filtrate collected in a conical flask. The filtrate was boiled at 100 ^{O}C and allowed to cool. The cooled solution was then decanted to get the coconut milk.

2.2.5: Preparation of Coconut Water

The coconut was washed with clean water, broken gently with a hammer and the water collected into a clean container.

2.3: Experimental animals

Pooled milt from 5 male brood stocks of catfish aged 16-18 months old and weighing 1.25-1.60kg and measuring 52.4-54.0cm in length were used for this experiment. The brood stocks were selected based on history of the pond and physical examination of the genital papilla, redness and vascularization of the genital papilla were considered indicators for breeding soundness. The brood stocks were purchased from Iliayo farm Kubwa, Abuja.

2.3: Milt collection

Milt was collected by sacrificing the brood stocks. The sperm sacs were exteriorized and washed with normal saline. The sacs were dried with a filter paper. The weight and size was measured and recorded. Milt was extracted into 5ml plain sample tubes. 4ml of milt was collected from both the left and the right sperm sacs and was maintained at room temperature.

2.5: Pre- Dilution and Microscopic Examination for African Cat Fish milt

Volume of semen was observed and recorded. The colour was graded from creamy to watery (3 to 1). A drop of fresh semen was made on a pre-warm glass slide and covered with a warm cover slip. The sample was viewed under the microscope starting from x4 magnification to observe wave motion and then individual motility at x10 magnification. A dilution of 1 in 10 was made with a formal saline for determination of sperm concentration. Sperm concentration was done using a haemocytometer at x40 magnification.

The five diluents were gently rocked and dispensed into tubes of 5ml capacity. 0.5ml of extended milt was dispensed into test tubes. Comprising 9 tubes of the five various extenders. The tubes were labeled properly and were kept in a cooler before transfer to a fridge at 4^oC for further chilling and storage.

African catfish milt was diluted 1 in 20 by taking 0.5ml of fresh milt into 9.5ml of extender at room temperature. Dilution was made with the five extenders prepared via quail egg yolk extenders. The extenders used in the experiment include;

- Citrated water as control.
- Soya Milk

- Coconut water
- Coconut Milk
- Mixture (Soya milk and Coconut water)

2.6: Statistical Analysis of Data.

Data was expressed as Mean (±SEM). And was subjected to Analysis of variance (ANOVA) to compare the diluents. P values < 0.05 were considered significant. The statistical analysis was carried out by using SPSS version 20.

III. Results

Table 1: Phuyochemical constituents of the extenders

Qualitative Test	Coconut Water	Coconut Milk	Soya Milk
Tanin	+	-	-
Saponin	-	-	+
Alkaliod	+	+	-
Flavonoid	-	-	+
Terpeniod	-	-	-
Phenol	+	-	-
Steriod	-	+	+
Balsam	-	-	-
Resin	-	-	+
Tuterpene	-	-	-
Glycoside	+	+	+

KEY:

+ ……..Present

-…….Absent/below detection limit

Table 2: Baseline data for pre-dilution fresh pooled milt of African catfish

1	p^H	7
2	Mass activity	+++
3	Motility	98%
4	Color	Creamy
5	Volume	3.5ml
6	Sperm count	$4.1x10^9$/ml

Table 3: Sperm characteristics of Catfish (Day 0)

Parameters	**NaC**	**Soya Milk**	**Coconut Milk**	**Coconut water**	**Mixture**
Progressive motility(%)	75.0 ± 0.30	0.0 ± 0.00	00.0 ± 0.01	00.0 ± 0.00	95.0 ± 0.02
Mass activity	3.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.11	0.00 ± 0.00	3.00 ± 0.00
pH	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.12	6.00 ± 0.86	6.00 ± 0.10
Concentration $x10^9$/ml	88.0 ± 0.10	90.0 ± 0.85	78.0 ± 0.07[a]	81.0 ± 0.75	7.0 ± 0.18

[a] Different superscripts in a row represent significant differences ($p<0.05$) between the groups.

The result of sperm characteristics of Catfish (Day 0) clearly shows no significant difference ($p>0.05$) in progressive motility, mass activity and Ph of the diluents across the row. However, there was significant difference ($p<0.05$) in sperm concentration which was lower in coconut milk (78±0.07) and coconut water (81±0.75) when compared with NaC diluents (88±0.10) but higher ($p<0.05$) in soya milk (90±0.85) and mixture (97±0.18) diluents

Table 4: Sperm characteristics of Catfish (Day 1)

Parameters	NaC	Soya Milk	Coconut Milk	Coconut water	Mixture
Progressive motility(%)	00.0 ± 0.00	00.0 ± 0.00	90.0 ± 0.45	00.0 ± 0.00	90.0 ± 0.22
Mass activity	0.00 ± 0.00	0.00 ± 0.00	3.00 ± 0.40	0.00 ± 0.00	3.00 ± 0.00
pH	6.00 ± 0.01	6.00 ± 0.42	6.00 ± 0.98	6.00 ± 0.19	6.00 ± 0.14
Concentration $x10^9$/ml	53.0 ± 0.19	70.0 ± 0.15	68.00 ± 0.17[a]	56.0 ± 0.08[b]	88.0 ± 0.13

[a,b] Different superscripts in a row represent significant differences ($p<0.05$) between the groups.

The sperm characteristics of Catfish (Day1) shows no signs significant difference ($p>0.05$) in the progressive motility, mass activity and pH of the diluents across the row, but there was significant difference ($p<0.05$) in the sperm concentration of coconut milk (68±0.17) and coconut water (56±0.08).

Table 5: Sperm characteristics of Catfish (Day 2)

Parameters	NaC	Soya Milk	Coconut Milk	Coconut water	Mixture
Progressive motility(%)	70.0 ± 0.84	00.0 ± 0.00	00.0 ± 0.00	0.00 ± 0.00	70.0 ± 0.81
Mass activity	3.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	3.00 ± 0.17
pH	6.00 ± 0.00	6.00 ± 0.44	6.00 ± 0.18	6.00 ± 0.00	6.00 ± 0.09
Concentration $x10^9$/ml	38.0 ± 0.08	75.0 ± 0.41[a]	40.0 ±0. 0[b]	33.0 ± 0.06[c]	71.0 ± 0.05

[a,b,c] Different superscripts in a row represent significant differences ($p<0.05$) between the groups.

The table above, shows no significant difference ($p>0.05$) in the progressive motility, mass activity and p^H of the diluents across the row. However , there was significant difference ($p<0.05$) in the sperm concentration of soya milk diluents (75±0.41), coconut milk diluents (40±0.10) and coconut water diluents(33±0.06)

Table 6: Sperm characteristics of Catfish (Day 3)

Parameters	NaC	Soya Milk	Coconut Milk	Coconut water	Mixture
Progressive motility(%)	65.0 ± 0.06[a]	00.0 ± 0.00	0.00 ± 0.00	00.0 ± 0.00	90.0 ± 0.05
Mass activity	2.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	3.00 ± 0.82
pH	6.00 ± 0.00	6.00 ± 0.12	6.00 ± 0.04	6.00 ± 0.00	6.00 ± 0.00
Concentration $x10^9$/ml	22.0 ± 0.15	40.0 ± 0.00	28.00 ± 0.00	20.00 ± 0.00	49.0 ± 0.04

[a] Different superscripts in a row represent significant differences ($p<0.05$) between the groups.

There was no significant difference ($p>0.05$) in the mass activity, pH and sperm concentration of the diluents across the row. Hence, there was significant difference ($p<0.05$) in the progressive motility of NaC diluents when compared with mixture diluents which was high

Table 7: Sperm characteristics of Catfish (Day 4)

Parameters	NaC	Soya Milk	Coconut Milk	Coconut water	Mixture
Progressive motility(%)	00.0 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	60.0 ± 0.04	70.0 ± 0.30
Mass activity	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.00 ± 0.00	3.00 ± 0.01
pH	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.17
Concentration $x10^9$/ml	12.0 ± 0.16	8.00 ± 0.02	7.00 ± 0.00[a]	10.0 ± 0.19	15.0 ± 0.25

[a] Different superscripts in a row represent significant differences ($p<0.05$) between the groups.

From the table4.6 above, it shows no significant difference ($p>0.05$) in the progressive motility, mass activity and pH of the diluents across the row, there was significant difference ($p<0.05$) in sperm concentration of coconut water diluents (7.00±0.00). There was no significant difference ($p>0.05$) in NaC diluents when compared with otherdiluents

Table 8: Sperm characteristics of Catfish (Day 5)

Parameters	NaC	Soya Milk	Coconut Milk	Coconut water	Mixture
Progressive motility(%)	60.0 ± 1.12	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	65.0 ± 0.05
Mass activity	2.00 ± 0.19	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.00 ± 0.00
pH	6.00 ± 0.21	6.00 ± 0.00	6.00 ± 0.11	6.00 ± 0.00	6.00 ± 0.08

Concentrationx10^9/ml	10.0 ± 0.18	12.0 ± 0.45	6.00 ± 0.77	3.00 ± 0.00 [a]	13.0 ± 0.10

[a] Different superscripts in a row represent significant differences ($p<0.05$) between the groups.

There was no significant difference ($p>0.05$) in the progressive motility, mass activity and pH of the diluents across the row. However, there was significant difference ($p<0.05$) in coconut water diluent (3±0.00) across the row for sperm concentration. Comparing NaC diluents with other diluents, there was no significant difference ($p>0.05$)

Table9: Sperm characteristics of Catfish (Day 6)

Parameters	NaC	Soya Milk	Coconut Milk	Coconut water	Mixture
Progressive motility(%)	30.0 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	40.0 ± 0.11
Mass activity	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.19
pH	6.00 ± 0.56	6.00 ± 0.00	6.00 ± 0.13	6.00 ± 0.08	6.00 ± 0.12
Concentration x10^9/ml	11.0 ± 0.98	2.00 ± 0.10[a]	11.0 ± 0.00	0.00 ± 0.00	13.0 ± 0.44

[a] Different superscripts in a row represent significant differences ($p<0.05$) between the groups.

The result of Day 6 shows sperm characteristics of Catfish showing no significant difference ($p>0.05$) in the progressive motility, mass activity and pH of the diluents across the row. However, there was significant difference in sperm concentration of soya milk diluent (2±0.10) when compared with other diluents across the row.

Table 10: Sperm characteristics of Catfish (Day 7)

Parameters	NaC	Soya Milk	Coconut Milk	Coconut water	Mixture
Progressive motility(%)	10.0 ± 0.00[a]	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	20.0 ± 0.34
Mass activity	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
pH	6.00 ± 0.62	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.13
Concentration x10^9/ml	00.0 ± 0.00	7.00 ± 0.00	0.00 ± 0.00	11.0 ± 0.21	9.00 ± 0.88

[a] Different superscripts in a row represent significant differences ($p<0.05$) between the groups.

The table above shows the sperm characteristics of Catfish (Day 7), showing no significant difference ($p>0.05$) in mass activity, pH and sperm concentration of the diluents. However, there was significant difference which was low ($p>0.05$) in mass activity, pH and sperm concentration of the diluents. However, there was significant difference which was low ($p>0.05$) in NaC diluent (10±0.00) when compared with mixture (20±0.34)

Table 11: Sperm characteristics of Catfish (Day 8)

Parameters	NaC	Soya Milk	Coconut Milk	Coconut water	Mixture
Progressive motility(%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	10.0 ± 0.00
Mass activity	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
pH	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.10	6.00 ± 0.35	6.00 ± 0.01
Concentration x10^9/ml	2.00 ± 0.00[a]	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	9.00 ± 0.76

[a] Different superscripts in a row represent significant differences ($p<0.05$) between the groups.

The sperm characteristics shown above shows no significant difference ($p>0.05$) in the progressive motility, mass activity and Ph of the diluents across the row but there was significant difference ($p<0.05$) in the sperm concentration with mixture which was low ($p>0.05$)

Table 12: Sperm characteristics of Catfish (Day 9)

Parameters	NaC	Soya Milk	Coconut Milk	Coconut water	Mixture
Progressive motility(%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mass activity	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
pH	6.00 ± 0.11	6.00 ± 0.57	6.00 ± 0.10	6.00 ± 0.45	6.00 ± 0.03
Concentration x10^9/ml	0.00 ± 0.000.	00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	3.00 ± 0.00

The table above shows no significant difference ($p>0.05$) in the progressive motility, mass activity, Ph and sperm concentration across the row.

IV. Discussion

Pituitary function and gonadotropic activity was evidenced in the super quality of semen (milt) obtained from base line semen parameters. It is known that gonadal function depends on the influence of endocrine production by the pituitary gland, specifically Follicle Stimulating Hormone (FSH) and luteinizing hormone (LH). Baseline semen parameters confirmed that the pituitary gland of the used male brood stocks were loaded with gonadotropic hormones without which sperm production and semen parameters would have been in the negative. High percentage motility coupled with creamy color and adequate semen volume of the male samples were confirmation that pituitary –gonadal axis were intact the catfish samples used. It was also observed that longevity during storage of chilled milt of African Catfish diluted in different extenders was up to 7 days as seen in citrated egg yolk and mixture groups. There was still elements of motility in those groups at that time. Storage motility up to 7 days was a super performance for the extenders used but primarily giving credence to the milt quality abinitio which was a reflection of pituitary function in African catfish. In most other species semen storage under chilled condition in various media last for about 4 days (Nagasa, 1954). From our findings, the longevity advantage observed in citrated egg yolk extender might be attributed to its cryoprotectant potentials and this disagrees with report the r eport that egg yolk has cryoprotectant antagonists, inconsistent composition and egg yolk granules that interfere with sperm motility and visibility (Ansari *et al.,* 2010). The mixture of coconut water and soya milk was also favoured. Following dilution, sperm concentration in soya milk was significantly high on Day 1 and significantly decline down the days which could be due to presence of genistein and saponin present as constituents of the soya milk enhancing visibility. Such components were not in coconut milk instead the milk based diluents were rich in fat globules obscuring sperm cells from being counted .due to poor optical clarity. Coconut water and citrated egg yolk (NaC) which have antioxidant properties may explain why the mixture which involved the coconut water and soyabean and citrated egg yolk lasted till 7 days with visble motility. This supports the publication of Sierens, 2002 who reported that In vitro isoflavones supplementation reduces hydrogen peroxide-induced DNA damage in sperm. Citrated egg yolk and the mixture of soya milk + coconut water proved to be more potent semen extenders for African catfish. It was concluded that by inference pituitary extraction of the African catfish could substitute endogeneous and exogeneous gonadotropins in estrous synchronization protocols in mammals including domestic ruminants. Citrated egg yolk and the mixture were most favourable semen extenders for the catfish. Hence, it was recommended that further studies be done in the application of the gonadotropin of pituitary extract origin in synchronization protocols in domestic animals.

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