

COMPARATIVE EFFECTS OF TRIS EGG-YOLK ORANGE JUICE EXTENDER ON HEAVY AND LIGHT STRAIN COCKS SEMEN DILUTED AT DIFFERENT RATE

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ABSTRACT

Dilution of neat semen is advantageous, as more hens can be inseminated from a single ejaculate semen sample. This study was conducted to evaluate the optimum dilution rate of marshal and Isa brown cocks semen diluted with Tris Egg-yolk Orange Juice extender Preparation of TEY-O extender was done, and maintained at 37°C. Ten marshal and Isa brown of 30-40 weeks of age from Obasanjo farms limited were used. Pooled ejaculates of 10 cocks from each strains were collected into glass funnel per trials. Blood stained and contaminated semen was discarded. Semen was examined for individual progressive motility. The extender and semen were kept at 37°C 5-10 mins before dilution. Pooled semen with motility > 80% was processed for dilution. Ejaculated pooled Semen from each strains were divided into four equal parts consisting of eight treatments and diluted at different ratio with TEY-O extender; undiluted semen, diluted Marshal (1:2, 1:3 and 1:4) and Isa brown (1:2, 1:3 and 1:4) cocks semen. Diluted and undiluted semen samples were evaluated for motility, viability, membrane and acrosome integrity. A total of three trials were conducted. The Freshly diluted semen samples showed no significant difference ($P < 0.05$) in all parameters evaluated. However, in marshal extended semen three folds dilution (1:2) had highest value, while in Isa brown, four folds dilution had highest value in most parameters. It is therefore concluded that semen dilution rate does not have significant influence on strains variability (heavy and light) and that both strains respond similarly to the different dilution rate.

Key words: Extender, Semen, Dilution, Egg-yolk, Strains

INTRODUCTION

The technique of Artificial Insemination is getting popular in other poultry species other than turkey. Since last few decades, more farms in Nigeria now rely on A.I for their broiler breeders. For example, fertility in the broiler breeds continues to decline as males are selected for growth, A.I may become cost effective in broiler breeder management (Reddy, 1995). A.I in poultry expresses better fertility than natural mating (Saeki and Nagomi, 1964). Even when under natural mating 80–85% of eggs are fertile, fertility can be further increase by 5–10% simply through augmentation with A.I to the propagation program (Gee *et al.*, 2004). Semen dilution with an extender for poultry is important because poultry semen is viscous and highly concentrated, containing approximately six to twelve billion spermatozoa per ml for roosters and toms, respectively (Donoghue and Wishart, 2000). Dilution of neat semen irrespective of the strains variability (meat/egg) laying type is therefore advantageous in that more hens can be inseminated from a single ejaculate semen samples. However, Choice of semen extender is an important aspect of semen processing for A.I (Peterson *et al.*, 2007).

Several investigators have compared the composition of various diluents and summarized fertility data across studies (Howarth 1983; Bakst 1990; Bootswala and Miles, 1992). But, no standard diluent for poultry semen yet, and studies are so variable in experimental design which includes species and strains response, dilution rate, time of insemination, vaginal depth and frequency of AI, Storage period and number of spermatozoa inseminated and duration of fertility analyzed that discerning the benefits of different diluents is difficult. Glutamic acid is believed to be the most important chemical constituent of avian seminal plasma and it has become a standard component of extenders (Siudzinska and Lukaszewicz, 2008). Egg-yolk known to contain a considerable amount of glutamic acid, and vitamin E, an evident for its antioxidant potentials has been a major component of cattle and some other farm animal species diluent. Moreover, optimizing the potentials of egg-yolk by supplementing it with orange juice as a conjugate antioxidant source has been reported to enhance semen quality *in-vitro* and *in-vivo* and subsequently egg fertility percentage (Balogun, 2019).

Penfold *et al.* (2001) also reported a significant advantage of diluting freshly collected spermatozoa 1:1 in BPSE on sperm motility and progressive status regardless of temperature (24°C versus 4°C) while further dilution in BPSE (1:10 or 1:100) caused a transient increase and a rapid decrease in progressive motility. Thus, maintaining the quality of poultry sperm *in vitro* shortly before and during

insemination has been observed to require suspending it in a solution capable of preserving its fertilizing ability till the sperm is inseminated and probably in the hen oviduct. Thus, identifying the appropriate and optimum dilution ratio ditto strains response is paramount and may be an important step in achieving better diluted semen quality and subsequently good fertility.

MATERIALS AND METHODS

This study was conducted at Oyo State College of Agriculture and Technology, in Collaboration with Obasanjo farm Nigeria limited (OFN) at Igboora. Tris buffer was prepared by adding 3.785g of tris to distilled water, and the pH was adjusted to 7.2. Fresh domestic fowl eggs were collected from the farm and disinfected, the shell was broken and yolk was separated from albumen. 20% egg-yolk was vigorously mixed with Tris buffer in the beaker and finally 10% orange juice was supplemented into it and mixed very well. TEY-O extender was kept in the freezer for further use. Ten Marshal and Isa cock birds were used from Obasanjo Farm Nigeria limited. Pooled semen with motility > 80% from each strains were separately simultaneously processed for dilution. Ejaculated pooled Semen from each strains were divided into four equal parts consisting of eight treatments and diluted at different ratio with TEY-O extender; undiluted semen, diluted Marshal (1:2, 1:3 and 1:4) and Isa brown (1:2, 1:3 and 1:4) cocks semen. The diluted and undiluted semen samples were evaluated for motility, viability, membrane and acrosome integrity. A Total of three trials were conducted, all data collected were subjected to analysis of variance and means were separated with Duncan multiple range test.

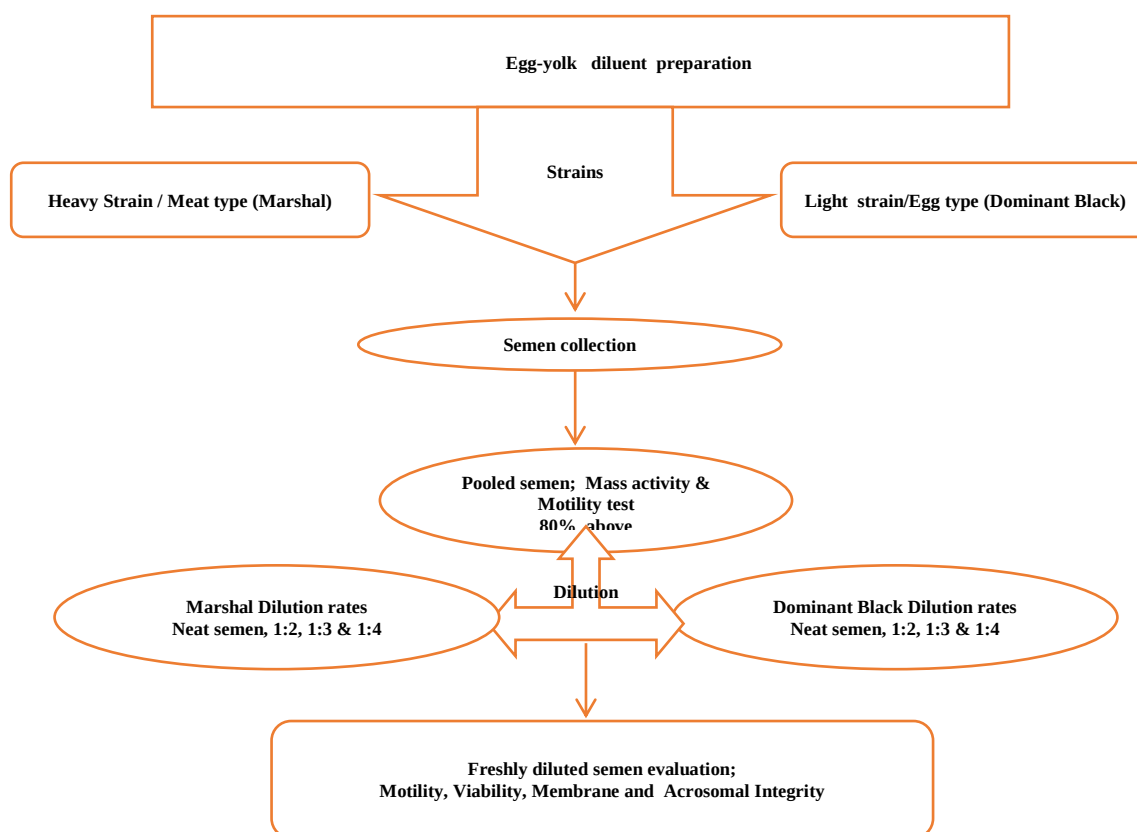


Fig1: Flow Showing Standardization of Semen Dilution Rate of Heavy and Light Strains Domestic Fowl With Tris Egg-Yolk Orange Juice Extender

Result and Discussion

Microscopic semen quality parameters of neat rooster semen extended with Tris egg-yolk orange juice extender is presented in (Table1). Freshly diluted semen samples showed no significant difference ($P < 0.05$) in most parameters evaluated such as percentage motility, viability, membrane integrity and acrosome integrity. However, in marshal extended semen, three folds dilution (1:2) had highest value in most parameters such as motility (93.33%), viability (95.00%) and membrane integrity (93.47%) while in Isa brown, four folds dilution (1:3) had highest value in most parameters; motility (91.67%),

viability (94.33%) and membrane integrity (93.33%). This result revealed that most microscopic semen parameters are not significantly affected by dilution rate with TEY-O extender on semen of both heavy and light strain cocks. Moreover, extender do not had adverse effects on immediate dilution with neat semen irrespective of the dilution rate up to four dilution folds. Similar results were reported by (Balogun et al., 2017a, Balogun 2019) on standardization of egg-yolk concentrations for rooster semen dilution and preservation. Also, the result obtained is in agreement with the previous studies in turkey semen (Iaffaldano *et al.*, 2010; Venkatesh 2005). However, Iaffaldano *et al.*, 2010 also reported superior spermatozoa motility with BPSE and IMV diluents at lower dilution (1:2 and 1:3), in which we also recorded highest values in our present experiment. Furthermore, Keerthy *et al.* (2016) buttress the result of our present study by observing that dilution rate did not showed any significant difference even between storage periods irrespective of the extenders and the guinea fowl varieties.

Table1: Effects of Different Dilution Rate on Heavy and Light Strains Cock Semen Extended With Tris Egg-Yolk Orange Juice Extender

Strains									
	Marshal Cock				Isa Brown				
Parameters	T1(neat semen)	T2(1:2)	T3(1:3)	T4(1:4)	T5(Neat semen)	T6(1:2)	T7(1:3)	T8(1:4)	SEM
Motility (%)	91.67	93.33	91.67	88.33	91.67	91.67	91.67	90.00	0.54
Viability (%)	92.67	95.00	93.33	91.33	95.00	92.67	94.33	90.33	0.52
Membrane Integrity (%)	93.38	93.47	93.17	90.33	91.50	91.07	93.33	88.33	0.57
Acrosome Integrity (%)	96.17	95.50	95.77	92.80	93.00	94.43	93.67	94.00	0.45

SEM : Standard Error of Mean

CONCLUSION AND RECOMMENDATION

It is therefore concluded that semen dilution rate does not have significant influence on strains variability and that both heavy and light strains cock semen responds similarly to different dilution rate with Tris egg-yolk diluents and can be diluted up to five folds for immediate artificial insemination use without exposing the sperm cells to any deleterious effects.

REFERENCES

- Bakst, M.R., (1990). Preservation of avian cells. In: Crawford, R.D. Ed. , *Poultry Breeding*. Elsevier, Amsterdam, Pp. 91–108.
- Balogun A S. (2019). Evaluation of antioxidant potentials of tomato & orange juices on extended poultry semen preserved with egg-yolk & coconut water diluents to improve selection intensity Ph.D Dissertation.
- Balogun, Adedeji Suleimon., Shivkumar., Mustapha Abdul-Rahman., Fatola, Olabamiji Sunday Gabriel and NidhaImtiyaz (2017a). Spermogram and Fertility Assessment of Cocks Semen, Extended with Coconut-water Extender Supplemented with Garlic Extracts. book of proceedings of IAHA Pre-Conference on Organic Animal Husbandry on the “Role of Livestock in Sustainable Agriculture” 6 - 8 Nov 2017 in Ghaziabad linked to 19th Organic World Congress of IFOAM in India. Pp. 164-169
- Bootsvala, S.M., Miles, R.D., (1992). Development of diluents for domestic fowl semen. *World's Poultry Science Journal*. 48, 121–128.

- Donoghue A M and Wishart G J. (2000). Storage of poultry semen. *Animal Reproduction. Science*, **62**:213–232.
- Gee GF, Bertschinger H, Donoghue AM, Blanco JM and Soley J. (2004). Reproduction in non-domestic birds: physiology, semen collection, artificial insemination and cryopreservation. *Avian Poultry Biology Review*. 15: 47–101
- Howarth B K. (1983). Comparison of diluents for holding cock semen six hours at 418°C. In *Poultry Science*, **62**:1084-1087.
- Iaffaldano N, Rosato MP, Manchisi A, Centoducati G, Meluzzi A (2010) Comparison of different extenders on the quality characteristics of turkey semen during storage. *Ital J Anim Sci* 4: 513-515.
- Keerthy AJ, Omprakash AV, Churchill RR, Hudson GH (2016) Effect of Semen Diluents, Dilution Rates, and Storage Periods on Spermatozoa Motility of Different Varieties of Guinea Fowl. *Journal of Veterinary Science Technology* 7: 378. doi: 10.4172/2157- 7579.1000378
- Penfold L, Harnal V, Lynch W, Bird D, Derricson S and Wildt E. (2001). Characterization of northern pintail (*Anas acuta*) ejaculate and the effect of sperm preservation on fertility. *Reproduction*, **121**: 267–275.
- Peterson K, Kappen M A P M, Ursem P J F, Nothling J O, Colenbrander B and Gadella B M. (2007). Microscopic and flow cytometric semen assessment of Dutch AI-bucks: effect of semen processing procedures and their correlation with fertility. *Theriogenology*, **67**: 863-871.
- Reddy R P (1995). Artificial insemination of broilers: economic and management implications. In: Bakst, M.R., Wishart, G.J. Eds. , Proc. 1st Int. Symp. on the Artificial Insemination of Poultry. Poultry Science Association, Savoy, IL, Pp. 73–89
- Saeki and Y and Nagomi Y (1964). Seasonal differences in fertility and hatchability of eggs produced by the natural mating and artificial insemination methods of chickens. *Japan Journal of Animal. Reproduction*. 10: 37-43.
- Siudzinska A and Lukaszewicz E 2008. Effect of semen extenders and storage time on sperm morphology of four chicken breeds. *J. Appl. Poult. Res*. 17: 101–108.