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Coenzyme Q10 and soyphosphatidylcholine in EK extender on preservation of Rhode Island Red poultry semen

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ABSTRACT

The objective of this study was to evaluate the efficacy of EK extender alone or incorporation with CoenzymeQ10 (CoQ10) and/or soyphosphatidylcholine (SPC) in poultry semen and their effects on seminal traits during temporal storage at 4^0C for different time intervals (12 h, 24 h, and 36 h). Heterospermic pooled semen samples diluted (1:4) with EK, EK + SPC, EK+ CoQ10 and EK + SPC + CoQ10 extenders separately, preserved and different spermiogram were assessed. Various seminal traits within the same extender differ significantly (p<0.05) among different groups and with different time intervals of storage. CoQ10 and SPC in the EK extender exhibited favorable synergistic effect on sperm quality and were able to protect the male gametes against cold-stress up to 36h at 4^0C. In this study, we concluded that incorporation of SPC and CoQ10 together in EK extender possess novel potentiality to maintain seminal quality during liquid storage of poultry semen at 4^0C and for their safe transportation and further use for Artificial Reproductive technologies (ARTs).

Keywords

CoQ10, SPC, EK Extender, Poultry Semen, Preservation

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INTRODUCTION

The genetic diversity within Indian indigenous chicken breeds is decreasing due to their replacement by high-producing exotic commercial hybrids, as well as selection for heavy broad-breasted broiler breeder strains has led to a continuous decline in fertility using natural mating (Mc Daniel,1978). Hence, artificial insemination (AI) will have to be employed to protect the indigenous germ pool. As poultry semen is highly concentrated and is of low volume and spermatozoan motility and fertilizing ability of undiluted neat semen stored in vitro usually decreases within 1h of collection, therefore, to utilize AI successfully, evaluation of semen quality before and after storage as well as prior to insemination is very important (Reddy, 1995) including type of diluents used, storage techniques and temperature to avoid a reduction in sperm quality (Donoghue and Wishart, 2000; Dumpala et al., 2006). Moreover, assessment of semen quality characteristics of the poultry gives an excellent indicator of the reproductive potential and has been reported to be a major determinant of fertility and subsequent hatchability of eggs (Peters et al., 2004).

Several types of extenders have been formulated for temporal storage of poultry semen at 4^0C and its suitable transportation; however, most of the researchers have reported maximum 24 h of preservation at 4^0C. (Sexton, 1978, 1988). For the confirmation of long time *in-vitro* survival of poultry spermatozoa, use of low temperature along with buffered saline having glycolytic substrates and few intermediates of citric acid cycle are not sufficient. Lipids are considered to have a vital role in conserving structure and function of spermatozoa both *in-vivo* and *in-vitro* conditions (Parks and Lynch, 1992; Jones, 1998). Lipids appeared to be greatly involved in *in-vitro* semen storage by cooling without freezing; the membrane lipid moieties in a liquid-crystalline phase may affects both physical and biochemical properties of spermatozoa (Parks and Lynch, 1992). Endogenous phospholipids are metabolized during *in-vitro* storage

of mammalian spermatozoa (Scoot and Dawson, 1968; Neill and Masters, 1972). Polyunsaturated fatty acids (PUFA) present in cell membrane may also be sensitive to lipid peroxidation (LPO) *in-vitro*. The susceptibility of Turkey and chicken spermatozoa to *in-vitro* LPO and positive effects of antioxidants on the success of semen preservation has been reported by many workers (Fujihara and Haworth, 1978; Blesbois et al., 1993; Wishart, 1984; Donoghue and Donoghue, 1997). However, the use of CoQ10 a potent antioxidant and sperm mitochondrial stabilizer along with SPC, the phospholipids and a good emulsifier and capable to maintain structural integrity of the cells have never been studied during hypothermic storage of poultry semen so far. Hence, the basic knowledge of using CoQ10 and SPC in the extender could be extremely useful for *in vitro* storage of poultry semen at 4^0C.

MATERIALS AND METHODS

Ethics: Ethical consent was obtained from the Animal Ethics committee from the Faculty of Veterinary and Animal Sciences at the West Bengal University of Animal and Fishery Sciences before this study was conducted.

Management and grouping of birds: Twenty eight healthy RIR cocks of forty-one weeks of age were divided into four groups (Gr I, II, III and IV) having seven cocks in each group. Individual groups of cock were reared separately under deep liter system. All the birds were kept in uniform husbandry practices, water was supplied *ad libitum* and received 14 h of light/day throughout the experiment.

Preparation of the extender:
Extender 1: EK extender was prepared as developed by Lukaszewicz (2002).
Extender 2: EK extender with CoQ10: EK extender 100 mL+CoQ10 30 mg.
Extender 3: EK extender with SPC: EK extender 100 mL+SPC 10 mg.
Extender 4: EK extender with CoQ10 and SPC: EK extender 100 mL+CoQ10 30 mg+SPC 10 mg.
All the prepared media were passed through Millipore filter separately to free the microbial contamination. Osmomolality and pH were adjusted to 330mOsm and 7.1, respectively. Prepared extenders were kept in sterile amber coloured coded bottle stored at 4^0C and used within a week.

Semen collection, dilution and evaluation: The cocks were adapted for semen collection within two weeks using abdominal sexual massage technique (Burrows and Quinn, 1937). Semen samples were collected in every five days interval in the afternoon (1 pm - 2 pm) for a period of five months. The birds responded to massage by partial aversion of cloaca, and semen samples were collected from the Ventral lip of the vent in sterile 15 mL graduated tubes in a thermo flask. Collected semen samples from each individual group was pooled as one sample and divided into two parts. Evaluations of the seminal traits were performed with one part of the heterospermic pooled neat semen samples collected from each group. Observation of spermatozoan progressive motility were assessed by standard subjective ranking method (WHO, 1997) using a phase-contrast microscope (×400).

Motility was expressed as the % of motile sperm with moderate to rapid progressive movement (Cheng et al., 2002). Only samples showing highest motility (>70%) were considered. Percentage of live spermatozoa was evaluated with 10 µL of fresh semen and 200 µL of Eosin-Nigrosin stain and smears were made with the semen-stain mixture on microscopic slides and examined under the microscope (x1000) (Lukaszewicz et al., 2008). Spermatozoan abnormalities were determined by 3% Rose Bengal stain prepared in 3.55% sodium citrate solution. Smears of semen samples were prepared on dry microscopic glass slides and allowed to air dry. Smears were placed on the staining rack, flooded with 3% Rose Bengal stain and allowed for 10 min. After staining, the stained Smears were washed by dipping into distilled water twice into two beakers for 3 sec each. Excess water was blotted carefully and the slides were washed and allowed to air dry. Gross observations of different types of sperm abnormalities in respect of head, neck, midpiece and tail were made under the microscope(x200; x400) (Tomar, 1976). Hypo-osmotic swelling test (HOST) was conducted and different types of sperm tail coiling were evaluated following the method of Ravell and Mordoe (1994). HOS solution was prepared with fructose (1.351 gm) and trisodium citrate (0.735 gm) in 100 mL. of double distilled water. Osmomolality was adjusted to 150 m O sm. Each 100 µL semen sample was mixed with 1 mL of HOS solution into separate round bottom test tubes and incubated at 37°C for 60 min providing 5% CO_2 in the air. After incubation, 20 mL of sample from each test tube was placed on clean glass slides and covered with cover slips. Swelling of different parts of sperm specially tail portion as positive response to HOS solution was observed under phase-contrast microscope (x200; x400). For each observation, 200 individual sperm cells from each sample in triplicate

were considered and their mean values were expressed as %.

The other part of the semen samples from individual groups was splited again into 4 equal parts (total 16 samples). The semen samples and extenders were kept at 35±2^{0}C prior to dilution. The dilution of each semen sample from the 4 groups were performed with prepared extenders separately at 1:4 ratio (0.25 mL semen: 1 mL of extender) in different 10 mL sterile coded glass tubes (Lake, 1960). Spermatozoan characters were evaluated again as above with 100 µL of extended semen samples immediately after dilution. Rest of the diluted samples stored at 4^{o}C in a refrigerator were used to determine the effects of different semen extenders on the male gametes at 12 h, 24 h and 36 h of storage respectively.

Statistical Analysis: Data were analyzed using SPSS package following standard procedures (Snedecor and Cochran, 1994) with Duncan's multiple range tests (Duncan, 1955).

RESULTS AND DISCUSSION

Seminal traits immediately after collection and just after dilution (0 h) are depicted in **Table 1**, where percentage of progressive motility, viability and HOS reactive spermatozoa differed significantly (p<0.05) among different groups, however, morphologically abnormal sperm remained non-significant among the groups when compared with the control.

Values (%) of sperm progressive motility (**Table 2**) viability (**Table 3**) and HOST reactive cells (**Table 5**) at 12 h, 24 h and 36 h exhibited significant differences (p<0.05) among different groups of extenders. The mean values (%) of morphological abnormalities (**Figure 1**) from different extender groups at 12 h, 24 h and 36 h had significant differences (p<0.05). The morphological abnormalities of spermatozoon were found highest in group I that contained EK extender only (**Table 4**).

Observations revealed that the Extender-4 which contained both the SPC and CoQ10 exerts maximum protective effect on spermatozoan motility, viability and HOS reactivity as well as able to reduce the number of spermatozoan abnormality significantly (p<0.05) at 24h and even up to 36 h of storage when compared with extender 1,2 and 3. Though the spermiogram were not optimum up to 36 h, however, extender-4 maintained its good protective effect.

Over the past 50 years, ARTs including preservation technologies have been developed for mammalian gametes and embryo which enables to run programmes to preserve genetic materials. Because of anatomical and physiological differences, preservation techniques of fowl sperm are less developed, specially as conditions vary between avian species and breeds within species and require advanced method of preservation. Additionally, semen has to be kept fertile in the female genital tract over several days or weeks after A.I requiring a higher vitality of stored/frozen-thawed semen as compared to mammalian spermatozoa. Hence, search for an optimal extender-composition for fowl semen is still in progress, because, differences in the extenders, additives and environmental conditions make it difficult to get variable estimations of the efficiency of various producers.

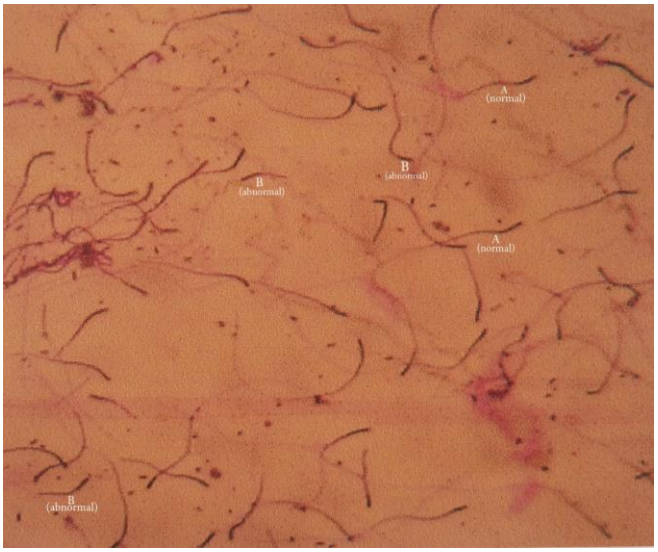

Figure 1. Microphotograph of RIR Cock sperm (normal A, abnormal B; Rose Bengal Stain, x 400).

Till date, in spite of using any extender, seminal characters diminished gradually with time during liquid storage of poultry semen, however, some important outcome from the present study have been achieved which could be explained by that the lipids are the main structural and functional components of the sperm and their compositions may undergo a series of modifications in relation to either physiologic events or from any stresses. Loss of phospholipids from sperm plasma membrane (SPM) occurs during cold shock (Parker and Graham, 1992). Chilling/freezing and thawing causes increase in reactive oxygen species (ROS) responsible for destruction of all cellular structure including membrane lipid and / or membrane bound PUFA resulting impairment of

cellular structure (Leinz, 2000). Chicken and turkey sperm contain high amount of polyunsaturated fatty acyl groups (Ravie and lake, 1985) and spontaneous peroxidation occurs during *in vitro* storage in both species (Cecil and Bakst, 1993). There are considerable evidences that such fatty acids play an important role in sperm function since causes of impaired fertility have been associated with reduced amount of these polyunsaturates in sperm cells (Kelso et al., 1996). However, the high degree of polyunsaturation typical to sperm lipids renders gametes highly susceptible to LPO, with the consequent risk of damage to cellular structures (Niki et al., 1993). Evidences indicate that the fatty acid composition of SPM, specially PUFA determine their biophysical characteristics, such as fluidity and flexibility both of which are necessary to fulfill specific functions including motility and fertilizing capacity of the sperm cells. Moreover, the high levels of PUFA render avian spermatozoa predispose to LPO and it seems reasonable that antioxidant protection plays a very crucial role in the maintenance of SPM integrity and thereby fertilizing

Table 1. Evaluation of pooled fresh semen samples (control) of RIR cocks and just after dilution with different extenders.

Extenders / Parameters	Control	Group-I (EK)	Group-II (EK+COQ10)	Group-III (EK+SPC)	Group-IV (EK+COQ10+SPC)
Progressive motility (%)	82.2±0.41^{a}	81.3±0.36^{ab}	80.8±0.38^{b}	80.7±0.36^{b}	81.2±0.32^{ab}
Viability (%)	90.8±0.62^{a}	87.3±0.51^{b}	86.1±0.65^{b}	87.1±0.70^{b}	88.2±0.74^{ab}
Morphological abnormality (%)	6.5±0.30	7.0±0.25	6.9±0.23	6.8±0.24	6.9±0.23
HOST (%)	69.6±0.30^{a}	64.3±0.53^{c}	67.2±0.32^{b}	65.2±0.57^{c}	67.1±0.31^{b}

Values expressed as Mean±SE
Mean values bearing different superscripts (a,b,c) in a row differ significantly (p<0.05)

Table 2. Comparison of progressive motility (%) among different groups (extenders) in different time intervals at 4^{o}C.

Extenders / Time (h)	Group-I (EK)	Group-II (EK+COQ10)	Group-III (EK+SPC)	Group-IV (EK+COQ10+SPC)
12	69.8±0.20^{ab}	72.5±1.54^{a}	63.9±0.50^{b}	75.5±1.17^{a}
24	51.5±0.76^{b}	55.9±1.95^{ab}	51.4±0.80^{b}	60.5±1.74^{a}
36	9.5±0.89^{c}	16.1±1.24^{b}	9.2±0.29^{c}	21.5±1.30^{a}

Values expressed as Mean±SE
Mean values bearing different superscripts (a,b,c) in a row differ significantly (p<0.05)

Table 3. Comparison of spermatozoan viability (%) among different groups (extenders) in different time intervals at 4^{o}C.

Extenders / Time (h)	Group-I (EK)	Group-II (EK+COQ10)	Group-III (EK+SPC)	Group-IV (EK+COQ10+SPC)
12	71.1±0.58^{c}	76.1±0.54^{b}	70.8±0.97^{c}	78.1±0.69^{a}
24	52.2±0.89^{b}	58.5±0.71^{a}	55.8±1.40^{ab}	59.8±0.96^{a}
36	20.2±0.90^{b}	25.8±2.15^{a}	25.2±0.95^{a}	24.5±1.38^{a}

Values expressed as Mean±SE
Mean values bearing different superscripts (a,b,c) in a row differ significantly (p<0.05)

Table 4. Comparison of morphological abnormalities (%) among different groups (extenders) in different time intervals at 4^{o}C.

Extenders / Time (h)	Group-I (EK)	Group-II (EK+COQ10)	Group-III (EK+SPC)	Group-IV (EK+COQ10+SPC)
12	9.2±0.24^{a}	8.4±0.16^{b}	8.9±0.27^{b}	8.5±0.16^{b}
24	29.3±1.17^{a}	16.4±0.63^{c}	19.3±0.51^{b}	16.7±0.73^{c}
36	44.9±0.99^{a}	27.9±0.79^{bc}	29.5±1.17^{b}	26.5±0.76^{c}

Values expressed as Mean±SE
Mean values bearing different superscripts (a,b,c) in a row differ significantly (p<0.05)

Table 5. Comparison of HOST positive sperm (%) among different groups (extenders) in different time intervals at 4°C

Extenders / Time (h)	Group-I (EK)	Group-II (EK+COQ10)	Group-III (EK+SPC)	Group-IV (EK+COQ10+SPC)
12	52.2±0.55^{c}	59.7±1.13^{b}	51.4±0.65^{c}	60.2±0.99^{a}
24	30.5±1.17^{b}	39.5±1.11^{a}	31.9±0.73^{b}	39.4±1.17^{a}
36	10.9±0.52^{c}	18.5±0.76^{a}	12.4±0.74^{b}	19.5±0.89^{a}

Values expressed as Mean±SE.
Mean values bearing different superscripts (a,b,c) in a row differ significantly (p<0.05) .

ability (Ladha, 1998). Hence, the Viability, storage efficiency and fertilizing ability of spermatozoa are highly dependent on the expression of an effective antioxidant capacity to these cells and in the surrounding seminal plasma. Exogenous lipids from the diluents have been shown strongly associated with the SPM and can prevent membrane damage during cold storage/cryopreservation and able to preserve successfully the spermatozoan motility, viability and fertility (Ricker et al., 2005).

The SPC contains three types of phospholipids namely phosphatidylcholine, Phosphatidyletholonamine and phosphatidylinositol, of which phosphatidylcholine plays a rate limiting role in the activation of numerous enzymes located in the membrane, for example, super oxide dismutase (SOD) and glutathione content (GSH). The SOD or GSH are important antioxidant in protecting cell membranes from damage elicited by reactive oxygen species (ROS). The ROS damages mitochondrial DNA causing reduced function of mitochondria. Lecithin may protect mitochondrial DNA ensuring the normal activity of mitochondria (Ulkowski et al., 2005). Hence, it is speculated that SPC is able to maintain the cellular structural integrity by protecting fatty acids from damages caused by ROS and lowering LPO to the cell membrane and stabilize SPM against adverse effects of cooling, freezing and thawing (Thun et al., 2002).

CoQ10, the ubiquinone, is a highly lipophilic component and is found in highest amount in the mitochondria and a powerful antioxidant that protects the body from free radicals (Weber et al., 1994). CoQ10 plays an important role in scavenging reactive oxygen intermediates and other free radicals and able to protect spermatozoa from membrane damage by inhibiting LPO (Lewin and Lavon, 1997; Duttan et al., 2000).

In sperm cells, the majority of CoQ10, an energy promoting agent could be concentrated in the mitochondria of the mid-piece; as a result, the energy for movement and all other energy dependent processes in the sperm cell depended on the availability of CoQ10. Lewin and Lavon (1997) reported an *in-vitro* study on 22 semen samples of asthenozoospermic men incubated with 50 µm of CoQ10 which significantly increased the sperm motility.

CoQ10 is the only lipid stable electron carrier in the mitochondrial electron transport system. The supplied energy is available in the form of ATP that was synthesized either by glycolysis in the cytoplasm (Ford and Rees, 1990), or through oxidative phosphorylation (OXPHOS) in the mitochondria (Mahadevan et al., 1997). ATP generation by OXPHOS in the inner mitochondrial membrane is transferred to the microtubules to derive motility. Hence, reduced motility may be associated with mitochondrial damage. Stress factors capable of uncoupling oxidation and phosphorylation in mitochondria, can be considered as possible effectors stimulating electron leakage and superoxide radical formation responsible for loss of spermatozoal membrane integrity, motility, livability and fertility. As CoQ10 is an integral redox and proton translocating components of the mitochondrial respiratory chain, plays a key role in energy metabolism and has potent antioxidative properties to protect cellular membrane integrity (Ernster and Dallner, 1995). Moreover, as CoQ10 is a lipophilic molecule, it is reasonably hypothyzed that CoQ10 can diffuse the phospholipids bi-layer of cellular membrane and thus protect the SPM.

Importantly, CoQ10 helps to preserve/recycle Vitamin E one of the major antioxidant of the cell membrane (Thomas et al., 1997) which also naturally present in, chicken sperm (Blesbois et al., 1993). Both CoQ10 and Vitamin C have been found capable to oxidize Vitamin E into a strong antioxidant (Beyer, 1994).

Developing a defense system against lipid peroxide damage is of practical importance in improving the extended liquid storage of RIR semen. Present study demonstrated improved survivability, motility, membrane integrity, concomitantly suitable reduction

in the percentage of abnormal sperm after cold storage of RIR semen with CoQ10 and SPC where both the component act as biological stabilizer of SPM and were able to scavenge ROS and LPO successfully from the SPM during hypothermic storage of semen at 4^0C and could be useful in the media across species.

CONCLUSION

The results of the present study provide a new approach that outlines a basic idea in the development for preservation of poultry semen at refrigerated temperature with modified EK extender containing CoQ10 and SPC for the first time as initial step. Based on the above findings , it is hypothesized that low temperature (4^0C.) would be better for long term storage of RIR poultry spermatozoa for more than 24 h when both CoQ10 and SPC were added together in EK extender. However, as the percentage of motility, viability, HOST positivity of spermatozoa was found declining with the extended period of holding time in the present experiment, it is now suggested that further study with different concentrations of CoQ10 and SPC may be conducted in order to get the best results.

REFERENCES

Beyer RE (1994). The role of ascorbate in antioxidant protection of biomembranes: Interaction with Vitamin E and Coenzyme Q10. Journal of Bio energy and Bio membrane, 26: 349-358.

Blesbois E, Grasseau I, Bloom JC (1993). Effects of vitamin E in fowl semen storage at 4°C. Theriogenology, 39: 771-779.

Burrows W, Quinn JP (1937). The collection of spermatozoa from the domestic fowl and turkey. Poultry Science, 26: 19-84.

Cecil H, Bakst MR (1993). *In vitro* lipid peroxidation of Turkey spermatozoa. Poultry Science, 72: 1370-1378.

Cheng FP , Guo TZ, Lin TE , Ursem PJF, Colenbrander B, Fung HP (2002). Annaul Variation in semen characteristics of pigeons (*Colombia Livia*). Poultry Science, 81: 1051-1056.

Donoghue A, Donoghue D (1997). Effects of water-and lipid – soluble antioxidants on turkey semen Viability, membrane integrity and motility during liquid storage. Poultry Science, 76: 1440-1445.

Donoghue AM, Wishart GJ (2000). Storage of poultry semen. Animal Reproduction Science, 62: 213-232.

Dumpala PR, Parker HM, Mc Daniel CD (2006). The effect of semen storage temperature and diluent type on the sperm quality index of broiler breeder semen. International Journal of poultry Science, 5: 838-845.

Duncan DB (1955). Multiple range and multiple F-tests. Biometrics, 11: 1-42.

Duttan PL, Ohnishi T, Darrouzet E, Leonard MA, Sharp RE, Cibney BR, Daldal F, Moser CC (2000). Coenzyme Q oxidation reduction reactions in mitochondrial electron transport. In: kagan VE, Quinn PJ (Edn.), Molecular mechanism in health and disease, CRC press, BocaRaton; pp 65-82.

Ernster L, Dallner G (1995). Biochemical, physiological and medical aspects of Ubiquinone function. Biochimica ET Biophysica Acta, 1271: 195-204.

Ford WCI, Rees JM (1990). The bioenergetics of mammalian sperm motility. In: Gagon C (ed.) Controls of sperm motility: Biological and Clinical aspects. CRC press, Boca Raton; pp 176-2002.

Fujihara N, Haworth B (1978). Lipid peroxidation in fowl spermatozoa. Poultry Science, 57: 1766-1768.

Jones R, (1998). Plasma membrane structure and remodeling during sperm maturation in the epididymis. Journal of Reproduction and Fertility (suppl.), 53: 73-84.

Kelso KA, Cerolini S, Noble RC, Sparks NHC, Speake BK (1996). Lipid and antioxidant changes in semen of broiler fowl from 25-60weeks of age. Journal of Reproduction and Fertility, 196: 201-206.

Ladha S (1998). Lipid heterogeneity and membrane fluidity in a highly polarized cell, the mammalian spermatozoon. Journal of Membrane Biology, 165: 1-10.

Lake PE (1960). Studies on the dilution and storage of fowl semen. Journal of Reproduction and Fertility, 1: 30-35.

Leinz A (2000). Lipoperoxidation damage of spermatozoan polyunsaturated fatty acids (PUFA): Scavenger mechanisms and possible scavenger therapies. Frontier of Bioscience, 5: 1-15.

Lewin A, Lavon H (1997). The effect of Coenzyme Q10 on sperm motility and function. Molecular Aspects of medicine, 18 (suppl): S213-219.

Lukaszewicz E (2002). Cryopreservation of *Anser anser* L. gander semen. Zeszyty Naukowe Akademii RoLniczej Wroclaw, *440*: 1-111.

Lukaszewicz E, Jersey A, Partyka A, Siudzinska A (2008). Efficacy of evaluation of rooster sperm morphology using different staining methods. Research in Veterinary Sciences, *85*: 583-588.

Mahadevan MM, Miller MM, Moutas DM (1997). Absence of glucose decreases human fertilizations in-vitro. Human Reproduction, 12: 119-123.

Mc Daniel GR (1978). Low fertility in broiler hatching eggs. Poultry Digest (Sep.), 12: 446-448.

Neill A, Masters C (1972). Metabolism of fatty acids by bovine spermatozoa. Biochemical Journal, *127*: 375-385.

Niki E, Noguchi EN, Gotoh N (1993). Polysaturated fatty acids in relation to sperm motility. Journal of Andrology, 15: 264-269.

Parker JE, Graham JK (1992). Effects of Cryopreservation procedures on sperm membranes. Theriogenology, 38: 200-222.

Parks J, Lynch DV (1992). Lipid composition and thermo tropic phase behavior of boar, bull, stallion and rooster sperm membrane. Cryobiology, 29: 255-266.

Peters SO, Omidiji EA, Ikeboi CON, Ozoje MO, Adebambo AO (2004). Effect of nacked neck and frizzled genes on egg traits, Fertilizing and hatchability in local chicken. In: self sufficiency of animal protein in Nigeria. Proceedings of the 9th Annual conference of Animal Science Association, Nigeria. Ebonyi State University, Nigeria; pp 262-264.

Ravell SG, Morde RA (1994) An Osmotic resistance test for bovine semen. Animal Reproduction Science, 36: 77-80.

Ravie O, Lake PK (1985). The phospholipids bound fatty acids of fowl and Turkey spermatozoa. Animal Reproduction Science, 9: 189-192.

Reddy RP (1995). Artificial Insemination of broilers: Economic and Management implications. In: Baskt MR, Wishart GT. (Eds.), Proceedings, 1st International symposium on Artificial Insemination on Poultry. Poultry Science Association. Savay, IL, 73-89.

Ricker JV, Linfar JJ , Delfino WJ, Kysar P, ACholtz EL, Tablin F, Crow JH, Ball BA, Meyers SA (2005) Equine sperm membrane phase behavior: The effects of Lipid based Cryoprotectants. Biology of Reproduction, 105: 046185-0461811.

Scoot T, Dawson R (1968). Metabolism of phospholipids by spermatozoa and seminal plasma. Biochemical Journal, 108: 457-463.

Sexton TJ (1978). A new poultry semen extender-3. Effect of Storage conditions on the fertilizing capacity of chicken stored at 5°C. Poultry Science, 57: 285-290.

Sexton TJ (1988). Comparison of commercial diluents for holding turkey semen 24 h at 5°C. Poultry Science, 67: 131-134.

Snedecor GW, Cochran GW (1994). *Statistical Methods*, 8th Edn., Oxford and IBH publishing Company, Kolkata, India.

Thomas SR, Neuzil JR (1997). Inhibition of LDL oxidation by ubiquinone -10. A protective mechanism for coenzyme Q in atherogenesis? Molecular Aspects of Medicine, 18: 585-103.

Thun R, Hurtado M, Janett f (2002). Comparison of Biociphos-Plus and TRIS-egg yolk extender for cryopresevation of bull semen. Theriogenology, 57: 1087-1094.

Tomar NS (1976). Artificial Insemination and reproduction of cattle and buffaloes. Saroj Prakashan, Katra-Allahabad, Uttar Pradesh, India; pp 60-82.

Ulkowski M, Musialik M, Litwinienko G (2005). Use of differential scanning colorimetry to study lipid peroxidation. I. Oxidative stability of lecithin and Linolenic acid. Journal of Agriculture and food chemistry, 53: 9073-9077.

Weber C, Jakobsen TS, Mortensen SA (1994). Antioxidant effect of dietary coenzyme Q 10 in human blood plasma. International journal of Vitamin and nutritional Research, 64: 311-315.

Wishart G (1984). Effects of lipid peroxide formation in fowl semen on sperm motility. ATP content and fertilizing ability. Journal of Reproduction and Fertility, 71: 113-118.

WHO (1997). WHO laboratory manual for the examination of human semen and semen-cervical mucus interaction. 3rd Edn. The Press syndicate of the University of Cambridge, Cambridge, London, UK.

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