Archivos de Zootecnia
Journal website: https://www.uco.es/ucopress/az/index.php/az/

Quality and fertility of rabbit semen diluted with watermelon juice

Jimoh, O.A.@ and Ayedun, E.S.

Agricultural Technology Department, Federal Polytechnic Ado Ekiti, Ekiti State, Nigeria.

Aditional Keywords

Conception rate.
Semen diluent.
Rabbit does.
Fruit.
Spermiogram.

SUMMARY

A study was designed to evaluate the suitability of watermelon juice as rabbit semen diluent and conception rate of does inseminated. Ripe watermelon (*Citrus lanatus*) was obtained and processed into juice using the standard procedure. A total of 10 rabbit male and 90 does, all crossbred New Zealand White x Chinchilla were used for the trial. Semen was collected from bucks using artificial vagina, and were diluted with watermelon juice at 1:0, 1:0.25, 1:0.67, 1:1.5, 1:4 semen to diluent and control 1:1 semen to normal saline and designated as T1, T2, T3, T4, T5 and T6; respectively. Semen analysis was conducted on fresh semen and the various diluted semen immediately at 37°C. Fifteen does each were randomly allotted to the different treatment in a completely randomized design and were inseminated with their respective treatment using the standard procedure. Conception rate, litter size and productivity index at birth were assessed at the end of the gestation period. Result obtained revealed that the range of values obtained for spermatozoa motility, structural membrane integrity, and acrosome integrity is within the accepted values for good quality semen. The conception rate of does inseminated with watermelon juice diluted semen at 1:1.5 was the optimal dilution rate with 75%. Litter size was significantly ($p<0.05$) highest (8.15kitts) at 1:0.25 dilution. The significantly ($p<0.05$) highest productivity index at birth was obtained in watermelon juice diluted rabbit semen at 1:0.25 and 1:1.5 inseminated does. In conclusion, it is revealed that watermelon juice is a potent semen diluent, and optimal dilution for rabbit semen is 1:1.5 and can be incorporated as extender constituent in the preservation of rabbit semen..

Palabras claves

Tasa de concepción.
Diluyente de semen.
Conejo hembra.
Fruta.
Espermiograma.

Calidad y fertilidad del semen de conejo diluido con jugo de sandía.

RESUMEN

Se diseñó un estudio para evaluar la idoneidad del jugo de sandía como diluyente del semen de conejo y la tasa de concepción conejos hembra inseminados. La sandía madura (*Citrus lanatus*) se obtuvo y procesó en jugo usando el procedimiento estándar. Para el ensayo se usaron un total de 10 conejo macho y 90 conejo hembra , todos de raza New Zealand White x Chinchilla. El semen se recolectó de los conejo macho utilizando una vagina artificial y se diluyó con jugo de sandía a una proporción 1: 0, 1: 0.25, 1: 0.67, 1: 1.5, 1: 4 o con solución salina normal 1:1; y se designó como T1, T2, T3, T4, T5 y T6 respectivamente. El análisis del semen se realizó en semen fresco y los diversos semen se diluyeron inmediatamente hasta 37°C. Quince de cada uno fueron asignar al azar para el tratamiento diferente en un diseño completamente al azar y se inseminaron con su tratamiento respectivo utilizando el procedimiento estándar. La tasa de concepción, el tamaño de la camada y el índice de productividad al nacer se juzgado al final del período de gestación. El resultado obtenido reveló que el rango de valores obtenidos para la movilidad del espermatozoide, la integridad de la membrana estructural y la integridad del acrosoma está dentro de los valores aceptados para el semen de buena calidad. La tasa de concepción de inseminación con semen diluido con jugo de sandía a una proporción 1: 1.5 fue la tasa de dilución óptima con 75%. El tamaño de la camada fue significativamente ($p<0.05$) más alto (8.15kitts) a una dilución 1: 0.25. El significativamente ($p<0.05$) mayor índice de productividad al nacer se obtuvo en semen de conejo diluido con sandía 1: 0.25 y 1: 1.5 inseminados. En conclusión, se revela que el jugo de sandía es un potente diluyente del semen, su dilución óptima para el semen de conejo es de 1: 1.5 y se puede incorporar como componente extensor en la preservación del semen de conejo.

Information

Cronología del artículo.
Recibido/Received: 06.12.2018
Aceptado/Accepted: : 26.02.2020
On-line: 15.04.2020
Correspondencia a los autores/Contact e-mail:
abubakarjimoh2011@gmail.com

INTRODUCTION

The most commonly used biotechnology in livestock farms in various developed and developing countries is Artificial insemination (AI). The advantages of AI include the use of high genetic merit progeny tested sires that are having some desired traits to an ample number of females. AI is a routine practice in rabbit production in developed countries (Alvariño, 2000). However, the practice in Nigeria is limited to higher institutions and research station. The technique offers significant benefits, including genetic selection, prolonged fertility even during unfavorable times of the year, cycle-based production, more efficient breeding programmes (Carluccio et al. 2004). The gains of artificial insemination have not been optimized by rabbit farms in Nigeria, due to unavailability of diluent and extenders for its practice partly due to the high cost of import for commercial diluents and extender. Ewuola et al. (2014) developed an improvised artificial vagina for use in local farms in Nigeria, to increase the ease of semen collection in rabbit and subsequently

reduced the time of on-farm artificial insemination in the rabbit. The propagation of the techniques and technical know-how and practice of AI in farms lies in the development of readily available diluents at low costs to production. The success of Artificial Insemination depends on many factors, such as the semen quality, insemination dose, time interval between semen collection and artificial insemination and depth of semen deposition in the female reproductive tract (Rriad et al. 2009). Fertility of rabbit bucks is of great importance because the sperm is responsible for fertilizing the ova (Hafez, 1960). Insemination with very small volumes may result in less effective mechanical drainage, while highly concentrated semen may be more irritating because of more contacts between spermatozoa and endometrium, resulting in intense inflammatory response (Araujo et al. 2013). Ewuola et al. (2014) reported that the dilution ratio above 1:2 ratio (semen: extender) reduced sperm concentration, declined sperm progressive motility and reduced mass activity. The significant role of diluents used for semen extension determines the success of artificial insemination (Jimoh 2019).

Spermatozoa are characterized by high proportions of polyunsaturated fatty acids (PUFAs) in the phospholipid fraction of their membranes (Khan et al. 2012). This characteristic composition confers to sperm plasma membrane the fluidity they require to undergo the membrane fusion events that characterize fertilization (Zaniboni et al. 2006). However, the high level of PUFAs increases the susceptibility of cells to free radical attack and lipid peroxidation (Balogun et al. 2016). Diluents for semen extension should possess the unique ability to protect the spermatozoa against oxidative damage. Antioxidants are believed to play a very important role in the body defense system against reactive oxygen species or free radicals and lipid peroxidation. Seminal plasma has a limited antioxidant capacity (Jimoh and Ewuola, 2018), the use of diluent having high antioxidant is required to maintain the viability and fertilizing capacity of spermatozoa. Recently, scientists are interested in the potential health benefits of phytochemicals and the synergistic effects of their multiple compounds compared to the single purified active fractions (El-Sheshtawy et al. 2017). Herbs and fruits play an important role in antioxidative defense against oxidative damage, possibly protecting the biological functions of cells (Shi et al. 2006). There is increasing interest in the protective and biological function of natural antioxidants contained in herbs, which are candidates for the prevention of oxidative damage (Jimoh et al. 2018). Fruits are good sources of natural antioxidants, containing many different antioxidant components (Cao et al. 1996). Watermelon (*Citrullus Lanatus*) contains large amounts of beta-carotene and is a significant source of lycopene (Mandel et al. 2005). Lycopene is of great interest because of its rich antioxidant and potential health benefits. Mangiagalli et al. 2007) suggested that lycopene may have beneficial effects on sperm motion characteristics as a result of its ROS-quenching abilities and protective effects against oxidative damage to structures crucial for the functional activity of male reproductive cells. Previous work of Jimoh (2019) reported that coconut water is a potent diluent for rabbit semen at 60% dilution rate. This study was designed to evaluate the potential of watermelon juice to serve as rabbit semen diluent for on-farm artificial insemination.

MATERIALS AND METHODS

The trial was conducted on 10 bucks and 90 does, all crossbred New Zealand White x chinchilla, aged between 9 and 14 months, housed in the rabbit farm of the teaching and research farm, department of Agricultural technology Federal Polytechnic Ado Ekiti. The farm consists of 3 shed which house the breeding does and bucks in a battery cage system. The rabbit shed is an open-sided house with natural lighting program (12D:12L), with the dynamic ventilation system. The experimental units are made of wire mesh double sided boxes of 1.1sq.m. The does were fed on non-medicated lactation pellet diet while the bucks were fed *ad libitum*.

Ripe watermelon (Citrus lanatus) sugar baby variety was purchased fresh from the market. The watermelon fruit was washed under running water, the watermelon was peel and sliced into bits. The watermelon juice was extracted using juice extractor (Mikachi Model No 1706). The juice was transferred into a test tube and centrifuge at 3000rpm for 15 minutes (Mediscan England 800D). The supernatant was thereafter referred to as watermelon juice, stored at -10^{0}C until required.

In the weeks previous to the experiment, semen samples were taken from all bucks to assess their characteristics, Sexual desire was measured in terms of reaction time in seconds and was estimated from the time the doe was placed inside the buck's cage up to the point when the buck ejaculated and suitability for reproductive purposes according to standard procedure, thereafter bucks of good semen quality were used. The semen was collected using an improvised artificial vagina produced according to Ewuola et al. (2014), made of a plastic cylinder with a latex liner secured around the rim, so that warm water could be placed between the cylinder and the latex liner. The artificial vagina was pre-warmed in water at 50ºC to 55°C, ensuring a temperature of 40ºC to 42°C at the time of collection, despite heat dispersion due to the small size of the device. Each ejaculate so collected was filtered through sterile pads to remove vesicle gland secretions which, being poorly soluble, could hamper the effectiveness of the dilution process, then examined macroscopically to assess volume. The ejaculates taken from each rabbit bucks were mixed to obtain a semen pool. Microscopic evaluation of the pooled semen was then performed using a compound microscope. Briefly, the parameters evaluated were:

Semen volume: Semen volume from each of the buck was measured using a tuberculin syringe to the nearest 0.1ml.

Semen Mass motility: A drop of semen was placed on a clean glass slide and examined with a microscope under x10 objective lens to determine mass activity. The mass activity was scored subjectively according to the intensity of the wave motion seen in the medium

by the collective activities of spermatozoa, from the absence of wave motion (+) to very turbulent motions (+++).

Sperm motility: A drop of semen with the aid of a micropipette was placed on a pre-warmed microscope slide and a drop of the diluent sodium citrate was added before it was covered with a glass coverslip and examine at a magnification of ×400. The percentage progressively motile spermatozoa were estimated, and score subjectively between 0 and 100. At least 5 microscopic fields were examined for each semen sample.

Sperm cell concentration: The sperm concentration was measured by the direct sperm cell count method, using an improved Neubauer haemocytometer slide. Formal saline was mixed with semen at v/v dilution. The diluted semen was then charged on each of two ends of the haemocytometer using a micropipette. The charged haemocytometer was placed on the microscope at a magnification of ×400. The concentration of sperm per volume was determined using the formula: C = 32,000 × N × D.

Where C = concentration of sperm cell per ml of semen, N = number of spermatozoa counted, D = dilution rate.

Structural membrane integrity: The structural membrane integrity of spermatozoa is an assay of liveability of spermatozoa. Thus it was determined by live to dead ratio of sperm cells. It involved adding a drop of the staining solution Nigrosin–Eosin on a clean slide and a drop of undiluted semen, mixed gently to prepare a smear. The slide was air-dried and examined with a microscope at x400 magnification.

Acrosome integrity: Acrosome integrity of the semen samples were examined by phase-contrast microscopy using x100 oil immersion objective after staining with Congo red and the slides were fixed with ethanol.

Total motile spermatozoa: The total motile sperm cell was calculated as the product of sperm concentration/ml and percentage motility of semen sample per animal.

Total live spermatozoa: Total live sperm cell was obtained by a multiple of sperm concentration per ml and percentage livability.

The heterospermic pool was divided into six samples, each diluted at different semen: diluent ratios for respective treatments.

Treatment 1- 1:0 Semen: watermelon juice

Treatment 2- 1:0.25 Semen: watermelon juice

Treatment 3- 1:0.67 Semen: watermelon juice

Treatment 4- 1:1.5 Semen: watermelon juice

Treatment 5- 1:4 Semen : watermelon juice

Treatment 6- 1:1 Semen: normal saline solution (Control)

Each of the six diluted samples was then further divided into two aliquots: one was used for *in vitro* evaluation, the other for *in vivo* test.

In vitro assay

The diluted semen samples were assessed at 37^0C and were assessed immediately for motility, structural membrane integrity, acrosome integrity, spermatozoa concentration, total motile and live spermatozoa as outlined above.

In vivo assay

AI of six groups of does (15/treatment), matched for age, weight, and parity were randomly allotted to the treatments in a completely randomized design. All receptive females (red colour of vulvar lips) were inseminated. The female is placed into a restraining box and inseminated with their respective diluted semen. Insemination was carried out with a curved glass pipette (0.5 cm diameter). To induce ovulation, females were treated with 8µg of Buserelin (T.P. Whelehan Son & Co. Ltd. Reg No. 1990 ESP) intramuscularly at the same time as insemination was performed. All females were inseminated with 10 million total spermatozoa, varying the inseminated volume depending on the sperm concentration. Gestation was checked by abdominal palpation 12 days after insemination was performed, and the gestation status was noted (pregnant or non-pregnant) for each female.

Parameters assessed were:

Conception rate: number of accomplished matings over the total number of pregnancies carried to term by the same female multiply by 100.

Litter size at birth: This is the number of kitts the doe kindles at birth.

Productivity index at birth: This is the product of litter size at birth and fertility rate.

Statistical Analysis

Data obtained were subjected to analysis of variance at $\alpha = 0.05$, the general linear model procedure of SAS, while means were separated using Duncan's multiple range test of the same software.

The statistical model is as the following:

$$Y_{ijl} = \mu + B_i + e_{ijl}$$

Where Y_{ijl} represents the value of semen characteristics and fertility indices measured in the l^{th} animal; µ is the overall mean for each character; B_i is the fixed effect of i^{th} watermelon juice dilutions at 5 rates and normal saline as control, and e_{ijl} is the random residual effect

RESULT AND DISCUSSION

Libido and semen characteristics of rabbit bucks used for this experiment (**Table I**) shows that the bucks were sexually active and fertile.

Effect of watermelon juice diluent on rabbit semen (**Table II**) reveal that sperm motility and acrosome integrity were influenced significantly ($p<0.05$) by the dilution ratio. Sperm motility of semen diluted with watermelon juice at T1, T2 and T6 were statistically ($p>0.05$) similar and significantly ($p<0.05$) lower values were obtained at higher dilutions of T3, T4 and T5.

Table I. Libido and semen characteristics of rabbit bucks (Características de la libido y el semen del conejo macho).

Semen characteristics	Mean	±SEM
Libido (sec)	7.50	1.04
Semen ejaculate volume (ml)	0.70	0.05
Semen mass motility (%)	93.33	4.01
Sperm motility (%)	94.30	1.27
Sperm cell concentration (x10^6 spermatozoa/ml)	58.69	2.49
Acrosome integrity (%)	89.04	2.22
Structural membrane integrity (%)	96.06	2.51

SEM: standard error of mean. (SEM: error estándar de la media).

Structural membrane integrity was similar across the dilution ratios. Spermatozoa acrosome integrity of semen diluted in T1 was significantly ($p>0.05$) similar to diluted semen on T6. The acrosome integrity of sperm cells on T2, T3, T4 and T5 share statistically ($p>0.05$) similar value. Sperm concentration, motile spermatozoa concentration and live spermatozoa concentration decreased apparently with the dilution ratios.

The role of watermelon juice as a diluent for rabbit semen is promising as revealed by the spermatozoa quality of the various dilution rates assessed. The range of values obtained for spermatozoa motility, structural membrane integrity, and acrosome integrity is within the accepted values for good quality semen. This demonstrates the ability of watermelon juice to retain the quality of the spermatozoa at these dilutions.

Citrullus lanatus is one of many plants that contain lycopene; which is of great interest because of its rich antioxidant and potential health benefits.

Similarly, Tvrda et al. (2017) suggest that lycopene supplemented to the semen extender acts as an effective motion-promoting and membrane protecting molecule, significantly improving sperm motility, membrane integrity, and mitochondrial activity during preservation. In agreement with this study is the report of Daramola et al. (2016) that supplementing extenders with fruit juice (orange and pineapple) at 10% consistently improved motility, acrosome integrity and membrane integrity, and reduced sperm abnormality. The improvement in semen quality could be attributed to the presence of substances in these fruits such as vitamins and phenolic compounds are known to function as antioxidants (Kiwon et al. 2003; Cutler et al., 2008).

Gardner et al. (2000) had earlier reported that concentrations of vitamin and total phenolic contents in fruit-juices have a strong relationship with antioxidant capacity (Daramola et al. 2016). Similarly, Amedu and Idoko (2016) reported that *Citrullus lanatus* promotes normal sperm morphology, sperm concentration, sperm volume and sperm motility in rat semen, probably due to the antioxidant activity of lycopene present, similar reports also corroborates this claims in chilled or frozen rabbit, turkey and fowl semen (Rosato et al. 2012). Mangiagalli et al. (2012) showed that the addition of lycopene to the drinking water of rabbit bucks increases the production of semen with volume and the total number of spermatozoa, and improves sperm kinetic characteristics and viability during semen storage at 5°C. Motility is an important parameter used for semen evaluation and Concannon and Battista (1989) suggest that at least 40%-50% sperm motility is necessary for success in artificial insemination. However, Linde-Forsberg and Forsberg (1989) postulated that 20%-30% sperm motility is necessary for pregnancy (El-Sheshtawy et al. 2017).

The trend of spermatozoa concentration in the various watermelon juice diluted semen reveal that the concentration declines with increasing dilution rate. Ewuola et al. (2014) reported a similar result in concentration and attributed to the dilution factor, due to an increase in the volume of seminal plasma when the sperm cells in the medium remain constant. Similarly, the higher the rate of dilution, the lesser the sperm cells in the semen sample (Kondracki, 2003) demonstrating an inverse relationship between dilution ratio and

Table II. Effect of watermelon juice diluent on sperm quality (Efecto del diluyente de jugo de sandía sobre la calidad de los espermatozoides).

	T1	T2	T3	T4	T5	T6	SEM	P Value
Sperm motility (%)	96.67^{a}	97.33^{a}	88.67^{b}	83.67^{b}	81.33^{b}	93.33^{ab}	4.83	0.00
Structural membrane integrity (%)	97.33	95.00	96.50	89.17	96.00	93.67	1.42	0.67
Acrosome integrity (%)	83.33^{a}	72.83^{b}	75.50^{b}	73.33^{b}	76.00^{b}	80.33^{ab}	1.70	0.09
Total spermatozoa concentration (x10^6 spermatozoa/0.2ml)	11.77	10.41	10.05	8.71	6.35	7.85	0.63	0.65
Motile spermatozoa concentration (x10^6 spermatozoa/0.2ml)	11.38	10.16	9.25	7.94	4.91	7o.33	0.30	0.32
Live spermatozoa (x10^6 spermatozoa/0.2ml)	11.07	9.70	10.03	8.51	5.83	6.86	0.28	0.46

ab means in the same row with different superscripts are significantly different. SEM: Standard Error of Mean. (ab significa que en la misma fila con superíndices diferentes son significativamente diferentes. SEM: error estándar de la media).

sperm concentration. Kommisrud et al. (2002) reported a decrease in the amount of seminal plasma increases the concentration of the spermatozoa. Semen dilution influenced the viability of spermatozoa in this study, and it is corroborated by Lahnsteiner et al. (2004) and Sadeghi et al. (2013) that increasing dilution ratio of semen leads to loss of protective effect thereby resulting in reduced sperm viability.

Fertility of does inseminated with watermelon juice diluted semen (**Table III**) show that does inseminated with T4 had highest value and least conception was obtained in does inseminated with T5. The litter size of does inseminated with T2 was significantly ($p<0.05$) highest and does inseminated T3 and T4 were statistically ($p>0.05$) similar and significantly ($p<0.05$) higher than does inseminated T1 and T6. The statistical ($p<0.05$) least litter size was obtained in does inseminated with T5. Productivity index at birth was significantly ($p<0.05$) highest in does inseminated T2 and T4. Does inseminated with T1, T3 and T6 had statistically ($p>0.05$) similar productivity index at birth and were significantly ($p<0.05$) higher than that obtained in does inseminated with T5.

The conception of rate of does inseminated with watermelon juice diluted semen at 1:1.5 was the optimal dilution rate. The result of this study agrees with the report of Ewuola et al. (2014) that dilution ratio above 1:2 ratio (semen: extender; using a conventional extender) reduced sperm quality, thus justifies the optimal dilution rate in watermelon juice. Similarly, Ewuola et al. (2017) dilution ratio of 1: 1 (semen: conventional extender) produced optimal conception when ovulation was induced with intramuscular administration of buserelin, conception rate of 85.71 %,71.43 % and 57.14 % which decreased as the ratio of semen to extender increased (1:1, 1:2, 1:3, respectively). The result of this study is higher conception rate (8.00-38.10%) and productivity index at birth (0.08-1.32) obtained by Jimoh and Ewuola (2018) in exotic rabbit breed artificially inseminated with semen diluted with conventional extenders at its standard dosage of 1:10 semen to an extender. Values of 75% conception rate obtained in T4 is within the range obtained by Roca et al. (2000) using frozen-thawed tris buffer extender for rabbit spermatozoa but similar litter size (6.79-7.89 kitt) compare to that of this study (8.15 kitts at 1:0.25 dilution). However, normal saline and undiluted semen inseminated does had lower values compared to dilution at 1:1.5, beyond this rate the fertility tends to decline. This showed the highest productivity at birth was obtained in watermelon juice diluted rabbit semen at 1:0.25 and 1:1.5 inseminated does.

Previous reports also corroborate the line of thought of this study; improved survival rate of spermatozoa preserved with tomato juice at 5°C was observed in African catfish (Clarias gariepinus; Adeyemo et al. 2007). In addition, Al-Daraji (2012) reported the protective effect of orange juice on spermatozoa against the harmful effects of lipid peroxidation in white layer cocks' semen. Daramola and Adekunle (2015) reported that supplementation of extenders with pineapple and cucumber juices during refrigeration of WAD bucks semen improved progressive motility, acrosome and membrane integrities, reduced abnormalities and MDA. The roles of natural antioxidants and sugars in these fruits as chemo-preventive agents against oxidative damage and energy sources for the sperm cells (Reza et al. 2011). The finding corroborated report that antioxidative compound was beneficial for sperm viability and reduction of lipid peroxidative damage to sperm membranes (Zheng and Zhang, 1997). The antioxidant potential of these fruit-juices with its structural characteristics of unsaturated side chain that has the ability to form resonance stabilized radicals (Marimuthu et al. 2007), together with the sugar content that provide energy and osmotic balance (Aboagla and Terada, 2004). Mangiagalli et al. (2012) reported that lycopene addition affects reproductive performance of multiparous rabbit does inseminated with fresh semen. Contrariwise, no significant contribution to laying hens inseminated with fresh semen from cockerels that received lycopene in drinking water (Mangiagalli et al. 2010) and in rabbit does inseminated with fresh and stored semen from rabbits fed alfa-tocopheryl acetate (Castellini et al. 2007). Likewise, Zaniboni et al. (2006) reported that reproductive performance features (hatchability rate, fertility, and embryo mortality) of female breeder turkeys were not affected after artificial insemination of semen enriched in vitamin E.

CONCLUSION

This study has revealed that watermelon juice is a potent diluent and can be incorporated as extender constituent in the preservation of rabbit semen. The use of watermelon juice as diluent for on-farm artificial insemination in rabbits is recommended at 1:1.5 (60%) dilution of semen to the extender for optimal conception.

Table III. Fertility of does inseminated with watermelon diluted semen (Fertilidad de conejos hembra inseminados con semen diluido en jugo de sandía).

	T1	T2	T3	T4	T5	T6	±SEM	P vlue
Conception rate (%)	50.00	50.00	50.00	75.00	25.00	50.00		
Litter size (number of kits)	2.95^{c}	8.15^{a}	4.05^{bc}	4.67^{b}	2.10^{d}	3.11^{c}	0.33	0.00
Productivity index at birth	1.50^{b}	4.00^{a}	2.00^{b}	3.50^{a}	0.50^{c}	1.50^{b}	0.21	0.00

abcd: means in the same row with different superscripts are significantly different. SEM: Standard Error of Mean. (abcd: significa que en la misma fila con superíndices diferentes son significativamente diferentes. SEM: error estándar de la media).

ACKNOWLEDGEMENT

The author appreciate the Teaching and Research Farm, Department of Agricultural Technology, Federal Polytechnic, Ado-Ekiti, Nigeria, for making available animals for the research. And grateful to Kolawole Richard, Osuolale Daramola, Kolade Adesola, Olonisaye Olufunke and Akinse Kemisola for the care and handling of the animals, and their resourcefulness to the study.

BIBLIOGRAPHY

Aboagla EME & Terada T 2004, 'Effects of egg yolk during the freezing step of cryopreservation on the viability of goat spermatozoa' *Theriogenology*, 62:1160-1172.

Adeyemo OK, Adeyemo OA, Oyeyemi MO, Agbede SA 2007, 'Effect of semen extenders on the motility and viability of stored African Catfish (*Clarias gariepinus*) spermatozoa' *Journal of Applied Science and Environmental Management*, 11:13-16.

Al-Daraji HJ 2012, 'Effect of diluents supplementation with different levels of orange juice on semen quality during liquid storage of roosters' semen' *International Journal of Veterinary Science,* 1:5-9.

Amedu NO & UP Idoko 2016, 'Dietary Consumption of *Citrullus lanatus* can ameliorate Infertility Potential of *Carica papaya* Seeds Extract in Male Rats' *British Biotechnology Journal* 11(2): 1-9,

Araujo, AG. Brandao, FZ, Guedes, MCA, Pinaffi, FLV, Silva, LA, Torres Filho, RA, Ferreira, AMR. 2013, 'Uterine blood flow evaluation after artificial insemination on different uterine sites in mares' *Animal Reproduction*; 10: 615.

Balogun AS, Jimoh OA, Mustapha A, Mohammed A, Akinosun AA & Ojo, JA 2016 'Assessment of different garlic extracts on fertilizing potentials of extended rooster semen used for artificial insemination' *Nigeria Poultry Science Journal; Vol 12 pp 84-91.*

Cao G, Sofic E & Prior RL. 1996. Antioxidant capacity of tea and common vegetables. *J Agric Food Chem,* 44:3426-3431.

Carluccio, A, Robbe D, De Amicis I, Contri A, Tosi U, Russo F & Paoletti M. 2004, 'Artificial Insemination in rabbits: laboratory and field trial with three different semen extenders' *World Rabbit Science*. 12: 65 – 79.

Castellini C, Mourvaki E, Dal Bosco A & Galli F 2007, 'Vitamin E Biochemistry and Function: A Case Study in Male Rabbit' *Reproduction in Domestic Animal* 42: 248-256. doi: 10.1111/j.1439- 0531.2006.00760.x

Concannon PW & Battista M. 1989, 'Canine semen freezing and artificial insemination' *In: Kirk RW, editor. Current veterinary therapy. Philadelphia*: W.B. Saunders Company

Cutler GJ, Nettleton JA & Ross JA 2008, 'Dietary flavonoid intake and risk of cancer in postmenopausal women: The Iowa Women's Health Study' *International Journal of Cancer,* 123:664-671.

Daramola JO, Adekunle EO 2015, 'Preservative effects of pineapple and cucumber juices on viability of refrigerated spermatozoa of the West African Dwarf bucks' *Pertanika Journal of Tropical Agricultural Science* 38:347-360.

Daramola, J.O., E.O. Adekunle, O.M. Onagbesan, O.E. Oke, A.O. Ladokun, J.A. Abiona, M.O. Abioja, I.K. Oyewusi, J.A. Oyewusi, O.A Isah, O.M. Sogunle & M.A. Adeleke 2016, 'Protective effects of fruit-juices on sperm viability of West African Dwarf goat bucks during cryopreservation' *Animal Reproduction*, v.13, n.1, p.7-13.

El-Sheshtawy, RI, El-Nattat, WS & GAD Ali 2017, 'Cryopreservation of cattle semen using coconut water extender with different glycerol concentrations' *Asian Pacific Journal of Reproduction* 6(6): 279-282

Ewuola E O, Omotosho GA & A. A. Adeyemi 2017, 'Effects of Buserelin acetate and semen extension on fertility in rabbit does under artificial insemination. Nigeria Journal of Animal Production 44(1):130 - 135

Ewuola, EO, Lawanson, AA & Adeyemi, A A 2014, 'An improvised artificial vagina for rabbit semen collection and the characteristics of the extended rabbit semen as panacea for artificial insemination' *Tropical Animal Production Investigation* 17 (1): 19-24

Gardner PT, Tamsin TC, White AC, Mcphail DB & Duthie GP 2000. The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit-juices. *Food Chemistry* 68:471-474.

Hafez ESE 1960, Sex Drive in Rabbits. *Southwest Veterinary*; 14, 46-49

Jimoh OA, 2019, 'Potential of coconut water to enhance fresh semen quality and fertility in rabbits' *Tropical Animal Health and Production* 45 (6) 1-7. DOI 10.1007/s11250-019-02011-z

Jimoh OA, Ihejirika UG, Balogun AS, Adelani SA & Okanlawon OO 2018, 'Antioxidant status and serology of laying pullets fed diets supplemented with mistletoe leaf meal' *Nigerian Journal of Animal Science*, 20 (1): 52-60.

Jimoh, OA & Ewuola EO 2018, 'semen characteristics, seminal biochemical and oxidative stress markers in rabbits during heat stress' *Journal of Veterinary Andrology* 3(2):35-44

Khan, RU, Rahman, ZU, Nikousefat, Z, Javdani, M, Laudadio, V & Tufarelli, V. 2012, 'Vitamin E: pharmaceutical role in poultry male fertility' *World Poultry Science*. Vol. 68: pp. 63-70.

Kiwon L, Yong JK, Hyong JL & Chang YL 2003, 'Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine' *Journal of Agriculture and Food Chemistry*, 51:7292-7295.

Kommisrud E, Paulenz H, Sehested E & Grevle IS 2002, 'Influence of boar and semen parameters on motility and acrosome integrity in liquid boar semen stored for five days' *Acta. Vet. Scand.* 4: 49–55.

Kondracki S 2003, Breed differences in semen characteristics of boars used in artificial insemination in Poland. *Pig News and Information.*24(4): 119–122.

Lahnsteiner F, Berger B, Horvath A & Urbanyi B 2004, 'Studies on the semen biology and sperm cryopreservation in the starlet, (Acipenser ruthenus)' *Aquaculture Research,* 35: 519–528, DOI: https://doi.org/10.1111/j.1365-2109.2004.01034.x.

Linde-Forsberg C & Forsberg M 1989, 'Fertility in dogs in relation to semen quality and the time and site of insemination with fresh or frozen *semen' Journal of Reproduction and Fertility* 39: 299-310.

Mandel H, Levy N, Izkovitch S & Korman SH, 2005, 'Elevated plasma citrulline and arginine due to consumption of *Citrullus vulgaris* (watermelon)' Berichte der deutschen chemischen Gesellschaft. 28(4): 467–472.

Mangiagalli MG, Cesari V, Cerolini S, Luzi F & Toschi I 2012, 'Effect of lycopene supplementation on semen quality and Reproductive performance in rabbit' *World Rabbit Science* 20: 141 – 148. doi:10.4995/wrs.2012.1150

Mangiagalli MG, Marelli SP & Cavalchini LG 2007, 'Effect of lycopene on fowl sperm characteristics during in vitro storage' *Archive fur Geflugelkunde*, 71, 25–29.

Mangiagalli MG, Martino PA, Smajlovic T, Guidobono, Cavalchini L & Marelli SP 2010, 'Effect of lycopene on semen quality, fertility and native immunity of broiler breeder' *British Poultry Science* 51: 152-157. doi: 10.1080/00071660903401540

Marimuthu S, Adluri RS, Venugopal PM 2007, 'Ferulic acid: therapeutic potential through its antioxidant property' *Clinical Journal of Biochemistry and Nutrition*, 40:92-100.

Reza A, Razi J & Hossein T. 2011. Influence of added Vitamin C and Vitamin E on frozen-thawed bovine sperm cryopreserved in citrate and tris-based extenders. *Vet Res Forum*, 2:37-44.

Roca, J, S Mart'ınez, JM Vázquez, X Lucas, I Parrilla & EA Mart'ınez 2000, 'Viability and fertility of rabbit spermatozoa diluted in Trisbuffer extenders and stored at 15°C' *Animal Reproduction Science* 64; 103–112, DOI: https://doi.org/10.1016/S0378-4320(00)00185-8.

Rosato MP, Di Iorio M, Manchisi A, Gambacorta M, Petrosino G, Centoducati G, Santacroce MP & Iaffaldano N 2012, 'In vitro survival and lipid peroxidation status of rabbit spermatozoa after both chilled and frozen storage in lycopene enriched extenders' *Livestock Science* 146, 199–202.

Rriad, RM, TST Seleem, KH El-Kholy & ZM Rawash 2009, 'Effect of sperm concentration and site of insemination on conception rate of rabbits' *Scientific Papers-Animal Science Series: Lucrări Ştiinţifice - Seria Zootehnie*, vol. 65. 47-51.

Sadeghi A, Imanpoor MR & Khalili M 2013, 'Effect Different Concentrations of DMSO and Dilution Rates on Motility Parameters of Stellate (Acipenser stellatus) Spermatozoa' *World Journal of Fish and Marine Sciences* 5 (4): 421–425.

Shi XJ 2006, 'Mistletoe alkali inhibits peroxi-dation in rat liver and kidney' *World Journal of Gastroenterology* 12(25): 4052- 4055.

Tvrda, E, A Mackovich, H Greifova, F Hashim & N. Lukac 2017, 'Antioxidant effects of lycopene on bovine sperm survival and oxidative profile following cryopreservation' *Veterinarni Medicina*, 62, (08): 429–436. doi: 10.17221/86/2017-VETMED

Zaniboni L, Rizzi R, Cerolini S 2006, 'Combined effect of DHA and α-tocopherol enrichment on sperm quality and fertility in the turkey' *Theriogenology*, 65: 1813-1827.doi: 10.1016/j.theriogenology.2005.10.013

Zheng RL & Zhang DL 1997' 'Effects of ferulic acid on fertile and asthenozoospermic infertile human sperm motility, viability, lipid peroxidation and cyclic nueleotides' *Free Radical Biology and Medicine.*, 22:58.