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Original research

Motility of rabbit buck semen extended with quail-egg yolk and turkey egg yolk citrate as rabbit buck semen extenders at refrigerated temperature

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ABSTRACT

Egg yolk from different avian species has been successfully used for the chill preservation of mammalian sperm but comparative efficacy in chill preservation of rabbit semen has not been previously evaluated. Fifteen (15) rabbit does and two (2) bucks were used for this study. Proximate analysis of the egg yolk of Quail (QEY), Turkey (TEY) and Chicken (CEY) was carried out using standard techniques. Semen was collected from the bucks using artificial vagina (AV) and evaluated for spermiogram. Semen was extended and stored at refrigerated temperature (5°C). Post extension motility was recorded for 5 days at 5°C. Fifteen (15) rabbit does were randomly assigned to 3 groups and synchronized using Lutalyse® (Dinoprost tromethamine), monitored for the display of oestrus signs and were subsequently inseminated using timed A.I. Pregnancy was diagnosed 12 days after insemination and conception rate was determined. Data generated was collated and presented using descriptive statistics. Proximate analysis of the egg yolks revealed relatively high ash content for TEY (4.2%), compared with QEY (1.5%) and CEY (1.2%) respectively. The fat extract for TEY (13.8%) was high when compared with QEY (10.7%) and CEY (12.6%) respectively. The carbohydrate content for CEY (1.2%) was high when compared with TEY, while QEY (0.6%) was the lowest. QEY had high CP when compared with CEY and TEY; 16.0% and 12.3% respectively, with TEY being the lowest. The moisture content (MC) of QEY was also very high (69.6%) when compared with CEY and TEY, 68.9% and 68.5% respectively. At room temperature, the forward progressive motility ranged from 95-99% for CEY, QEY and TEY respectively. Extenders containing CEY and QEY performed well in keeping the sperm cells alive within the first 24 hours; the progressive motility ranged from 80-90% and 80-95% respectively. The TEY extender had progressive motility ranged between 40 and 95%. Comparatively, the progressive motility of the spermatozoa recorded 48 hours post dilution for the CEY, QEY and TEY extenders are 15-35%, 40%, 15-20% respectively. Synchronized does displayed oestrus after 12 hours and those inseminated with extenders CEY, QEY and TEY were diagnosed pregnant (100%). It can be concluded therefore, that QEY has a better protective ability to maintain motility of refrigerated extended spermatozoa of rabbit bucks. Further studies are suggested to confirm the fertility success rate obtained in this study

Keywords: Avian eggs, semen, rabbit, extension, oestrus synch, artificial insemination, conception.

INTRODUCTION

The shortage of animal protein supply in Nigeria and other developing nations calls for exploration of other livestock species that are yet to play major role in animal protein supply to help fill the supply gap. Rabbit farming has been identified to have considerable potential in increasing animal protein supply and thereby reduce malnutrition and poverty (Oseni & Lukefahr 2014). Rabbits' short gestation period, rapid growth rate, high fecundity, small body size and their ability to utilize forage allows for low investment and early benefits that is possible with rabbit farming (Adedeji *et al*., 2015). Also, low sodium, fat and cholesterol level and high contents of polyunsaturated fatty acids, proteins and essential amino acids of rabbit meat (Dalle Zotte & Szendrő, 2011) makes it not only suitable for increasing animal protein supply but also for promoting healthy leaving. In order to achieve increased rabbit meat supply, modern rabbit husbandry techniques encompassing areas of disease management, improved feeding and genetic improvement have to be employed. Modern commercial rabbit meat production relies more on artificial insemination (AI) than natural breeding (Rosato & Nicolaia 2010). This is obviously due to the merits of AI over natural breeding which among others include allowing the breeding of large number of females at the same time using semen from few males with superior traits. Semen extension helps to increase the life

span of ejaculated spermatozoa and increases the number of females that can be bred with an ejaculate. Media used for semen extension dilute semen and preserve spermatozoa by being isotonic, serving as energy source, having buffering capacity, protecting against cold shock and controlling microbial contamination (Raheja *et al.,* 2018). Commonly, rabbit semen extenders are based on the combination of egg yolk and sodium citrate buffer (Nishijima *et al*., 2015, El-kalawy *et al*., 2012). Egg yolk serves to protect sperm cells from cold shock during cooling. Traditionally, the chicken egg yolk is commonly used for semen extension because of it wide availability. However, egg yolks from different avian species have different combination of fatty acids, phospholipids and cholesterol (Akhter *et al*., 2017). These variations in constituents of egg yolks can lead to difference in the protective effect they have on spermatozoa during cooling. There have been reports on compared protective effect of egg yolk from different species of birds on some mammalian spermatozoa. For example, Akhter *et al.* (2017) reported that quail and turkey egg yolk offer advantage over chicken egg yolk in the protection of buffalo semen. Santiago-Moreno *et al*. (2020) reported that quail egg yolk offers no advantages over chicken egg yolk in the cryopreservation of Spanish ibex epididymal spermatozoa. Kulaksiz *et al.* (2010) suggested that chucker egg yolk could be used as an alternative for chicken egg yolk in semen extender for cryopreservation of ram semen. To the best of our knowledge, reports are limited on in-vivo and in-vitro comparison of rabbit spermatozoa extended using egg yolks from different species of domestic birds during liquid storage. This study compared the preservative ability of egg yolk from quail, turkey and chicken in the preservation of rabbit semen during storage at 5^{o}C.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS AND THEIR MANAGEMENT

The study was conducted at the experimental farm of Theriogenology unit, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta (FUNAAB), Ogun State, Nigeria. Fifteen (15) matured rabbit does and two (2) rabbit bucks (mixed breeds) of 6-8 months old were used for this study. They were acquired from different farms located in Abeokuta and housed in hutches at the College of Veterinary Medicine, Federal University of Agriculture Abeokuta. All animals were tagged for ease of identification and screened by blood and fecal examinations for both ecto- and endo-parasites. Rabbits were acclimatized for a period of four weeks to ensure they were not incubating disease condition. They were intensively maintained on concentrate feed (Grower mash, Vital feed®, Nigeria) and forages. Clean water was provided *ad libitum* throughout the duration of the experiment.

PROXIMATE ANALYSIS OF THE EGG YOLK

The moisture, protein, fat, carbohydrate and ash content of the egg samples were determined using the methods of the Association of Official Analytical Chemists (AOAC, 1990).

PREPARATION OF BUFFER (2.9% SODIUM CITRATE)

The buffer, 2.9 % solution of sodium citrate was prepared by dissolving 2.9 g of sodium Citrate in clean sterile 100 ml-volumetric flask with distilled water and the solution was filled up to 100 ml mark. The solution was kept overnight in a dark cupboard.

PREPARATION OF THE DILUENTS

Fresh eggs of quail, chicken and turkey were disinfected with 70% ethyl alcohol and subsequently cracked and poured into an egg-yolk separator to separate egg yolk from the albumen. Egg-yolks were then centrifuged at about 4,000 revolutions per minute for 5 minutes.

COMPOSITION OF THE EXTENDERS

The composition of the extenders is as shown in Table I., Egg yolk from three different poultry species was used; the quail egg, turkey egg and chicken egg. The chicken egg yolk extender served as the control for this experiment. The other constituent of the extender include: sodium citrate buffer (2.9%) and antibiotics (2-3 drops of penicillin streptomycin).

TABLE I: Composition of the extenders

INGREDIENTS	**T1**(QEY)	**T2**(TEY)	**CONTROL**(CEY)
Egg yolk (%)(v/v)	20	20	20
2.9% Sodium citrate (%)(v/v)	80	80	80

Legend: QEY- quail egg yolk; TEY- turkey egg yolk and CEY- chicken egg yolk

SEMEN COLLECTION AND EVALUATION

Semen was collected from two rabbit bucks. The bucks were trained rained to adapt to ejaculate into artificial vagina (AV) during the four weeks of acclimatization using a female teaser as described by Morrell (1995). An improvised AV was assembled using syringes, plain sample bottles and condoms. The AV was pre-warmed in a hot water bath and hand-held beneath the teaser with the open end pointed in a caudal direction. As the buck begins to mount, the AV was

placed more caudally to allow penetration of the penis of the male rabbit into the AV and ejaculation occurred rapidly following penetration of the penis into AV (Naughton *et al*., 2003.). The ejaculates were collected and kept warm at a temperature of 37 °C using warm water.

Gross assessments of the ejaculates were done by evaluation of the color, volume and assessment for gross evidence of contamination, hemorrhage or inflammatory change. Mass activity was evaluated at 100× magnification using light microscope following placement of a drop of raw semen on a pre-warmed glass slide to observe the amount of wave motion. Individual motility was subjectively evaluated by diluting small quantity of raw semen on a pre-warmed slide with sodium citrate buffer and observing under microscope for forward progressive motility. Percentage livability (%) was assessed using one drop of semen mixed with warmed 2-3 drops of eosin-nigrosin stain on a warm slide as described by Wells and Awa (1970). A thin smear was made and air dried from the mixture of semen and stain. The live and the dead sperm cells were separately counted and the ratio of the live to dead sperm cells was calculated. Semen concentration was determined using the improved Neubauer haemocytometer as was previously described (Christensen *et al.,* 2005).

SEMEN EXTENSION

The two ejaculates were pooled and 0.5ml of the pooled ejaculate was dispensed into bottles containing 5mls of the different extenders (1:10 dilution) and stored at refrigerated temperature (5°C). Post dilution motility was recorded immediately and motility at refrigeration temperature was monitored for 5 days at an interval of 2 hours for the first 12 hours and thereafter was read at an interval of 12 hours.

ESTRUS SYNCHRONIZATION AND ARTIFICIAL INSEMINATION

Fifteen (15) rabbit does were synchronized using Lutalyse® (Dinoprost tromethamine) at the rate of 0.1ml per animal and were monitored for the display of estrus signs (tapping of the feet, moistened and oedematous vulva, red colour of the vulvar lips, mounting of each other), after which they were subsequently inseminated twice using timed A.I. with the same volume of extended semen (0.1ml) 12 hours after synchronization at 6 hours interval. Insemination was carried out with a 1ml insulin syringe. Gestation was checked by abdominal palpation 12 days after insemination. Pregnant and non-pregnant status was noted for each doe to evaluate for conception rate. From the result obtained, Conception rate was determined and expressed as percentage.

DATA ANALYSIS

Data generated was collated and presented using descriptive statistics.

RESULT

Semen was collected from two rabbit bucks for this study. Table II shows the pre-dilution ejaculate characteristics (spermiogram) of the semen. From this table, the semen volume ranges between 0.9 and 1ml; mass activity was between 3.0 and 4.0; percentage progressive motility 95-97%; percentage livability was between 80 and 90% and sperm concentration was between 1.05 x 10^9 and 1.15 x 10^9 sperm cells / ml respectively.

Table II: Pre-extension ejaculate characteristics of rabbit buck semen

EJACULATE CHARACTERISTICS	buck 1	buck 2
Volume (ml)	0.8	1.0
Colour	Milky	Milky
Mass Activity	3.0	4.0
% Progressive Motility	95	97
Percentage Livability (%)	90	80
Semen Concentration (sperm cells × 10^9/ml)	1.05	1.15

Table III shows the proximate analysis of all the 3 egg yolks. The ash content for the turkey egg yolk was relatively high, 4.2%, when compared with the quail and chicken egg yolk that had 1.5 and 1.2% respectively. The fat extract for the turkey egg yolk was high, 13.8% when compared with the quail and chicken egg yolk with 10.7% and 12.6% respectively. The carbohydrate content for the chicken egg yolk was also higher when compared with the turkey egg yolk, 1.2% while the quail egg yolk was the lowest with 0.6% carbohydrate content.

The crude protein for the quail egg was higher when compared with the chicken and turkey; 16.0% and 12.3% respectively, with the turkey being the lowest. The moisture content (MC) of the quail egg yolk was also very high (69.6%) when compared with the chicken and turkey egg

Table III: Proximate Analysis for Egg Yolks from Different Avian Species

Parameters	Quail egg yolk	Chicken egg yolk	Turkey egg yolk
ASH (%)	1.5	1.2	4.2
Fat Extract (%)	10.7	12.6	13.8
Carbohydrate (%)	0.6	1.3	1.2
Crude Protein (%)	17.6	16.0	12.3
Moisture Content (%)	69.6	68.9	68.5

yolk with MC of 68.9% and 68.5% respectively.

Table IV: Post extension motility of the rabbit semen in different extenders at refrigerated temperature (5°C)

Tag / Duration	CEY	QEY	TEY
0hr	96	99	95
2hrs	90	95	95
4hrs	85	90	70
6hrs	80	85	65
8hrs	90	90	50
10hrs	90	90	65
12hrs	80	90	70
24hrs	80	80	40
36hrs	35	40	20
48hrs	15	40	15
60hrs	15	40	15
72hrs	10	30	5
84hrs	15	40	5
96hrs	10	30	5
108hrs	0	20	5
120hrs	0	10	0

Legends:
CEY = Chicken Egg Yolk
QEY = Quail Egg Yolk
TEY = Turkey Egg Yolk

The results in Table IV above showed that at 37°C before storage at refrigerated temperature, the forward progressive motility (FPM) in the diluted semen ranges were 96%, 99% and 95% representing the extended semen with the exotic chicken egg yolk, quail egg yolk and turkey egg yolk respectively.

FPM in QEY extended semen was higher at 37°C while for the exotic chicken egg yolk and turkey egg yolk are similar.

The motility at 24 hours at refrigerated temperature was taken every 2 hours from the 0 hours to 24 hours for all the egg yolk. Extenders containing the chicken egg yolk and quail egg yolk performed well in keeping the sperm cells alive within the first 24 hours; the progressive motility ranges from 80- 90% and 80-95% respectively. The turkey egg yolk extender performed fairly well in keeping the sperm cells alive within the first 24hours; the progressive motility ranges between 40 and 95%.

From the motility result in Table IV, progressive motility decreased as the period of storage increased. Comparatively, the progressive motility of the spermatozoa recorded after the first 24hours for the various extenders varies from the ones taken at 12hours interval over a period of 120 hours. The evaluation done 48hours post dilution for the CEY, QEY, TEY extenders are 15-35%, 40%, 15-20% respectively. By 120 hours post storage at refrigerated temperature, all the sperm cells were found dead in CEY and TEY extenders except QEY extenders which showed 10% motility of the sperm cells, but are not viable again because the extenders was already contaminated with bacteria cells.

OESTRUS INDUCTION AND ARTIFICIAL INSEMINATION

All the does synchronized using $PGF_{2\alpha}$ (Lutalyse® Zoetis USA) at the rate of 0.1ml per animal displayed estrus after 12 hours and this lasted for a period of 24 hours. All does on heat were inseminated with the same quantity (1.0 ml) of the prepared diluents for the 3 groups, twice at 6 hours interval using extenders of the CEY, QEY and TEY respectively. Pregnancy was confirmed by abdominal palpation of the experimental animals 12 days after insemination.

All the does artificially inseminated with extenders CEY, QEY and TEY were diagnosed to be pregnant (Table V)

Table V: Conception rate post insemination of the rabbit does

EXTENDERS	Number of does inseminated (n=5)	Number pregnant	Conception rate (%)
CEY	5	5	100
QEY	5	5	100
TEY	5	5	100

DISCUSSION

From the result of the proximate analysis; the quail egg yolk has more cholesterol content than the turkey and chicken egg yolk, this observation is supported by the findings of Daramola *et al.,* (2013), who reported similarly that egg yolk from different avian species such as duck, quail, pigeon, chicken, and turkey has different combinations of fatty acids, phospholipids, and cholesterol. The quail egg yolk has a low fat content when compared with the turkey and chicken egg yolk from the result of the proximate analysis (QEY<CEY<TEY). The moisture content in QEY was higher when compared to CEY and TEY. Isah *et al.* (2015) stated that the moisture content of foods above 15% favours microbial growth which will result into food spoilage. Thus, since the moisture content of the various eggs exceeds 15% thus signifies that all eggs cannot be stored for long.

Egg yolk is one of the most commonly used cryproctectants during the preservation of extended semen. According to Akhter *et al.* (2017), the beneficial effect of egg yolk in the cryopreservation of sperm can be attributed to a resistance factor, which helps to protect the sperm against cold shock; and storage factor that helps to maintain viability. Routinely, the egg yolk is used as cryoprotectant that after disruption of the low-density lipoprotein fraction, release the

phospholipids that form a protective film at the surface of spermatozoa membrane. It has also been reported that phospholipids from egg yolk could merge with spermatozoa membranes and replace some phospholipids and thereby decrease their phase transition temperatures. It was also reported by Akhter *et al.,* (2017) that the cholesterol interacts with the phospholipid hydrocarbon chains at temperatures below the phase transition, forces the chains apart, making the membrane more stable. Interestingly, the sperm membranes of different species also vary in their cholesterol and phospholipid content that influences their susceptibility to cold shock. Therefore, the differences in sperm membrane composition and the components of the egg yolk from different avian species may culminate in species-specific interactions (Akhter *et al.,* 2017). Trimeche *et al.,* (2009) reported that quail egg yolk contained significantly more phosphatidylcholine, less phosphatidylethanolamine and a smaller ratio of polyunsaturated to saturated fatty acids than CEY. Turk and Barnett (1971) reported that duck and quail eggs were much richer in cholesterol than chicken eggs, although other researchers have shown similar levels of cholesterol between duck, quail and chicken eggs (Bair and Marion, 1978). This may be due to differences in the diets and breeds of the birds tested between studies. It has been suggested that proteins present in the LDL fraction of egg yolk may break down the lipoproteins, freeing the lipid and allowing it to interact with the sperm plasma membrane (Watson, 1981). Hence, the protein content of the yolks may have an important role in the protection against cooling damage and cryoprotection of sperm during freeze-thawing. More research is needed to identify the specific yolk proteins involved in this protection of sperm, in order to better understand the mechanisms by which yolk affords this protection, and perhaps improve the post-thaw quality of mammalian sperm.

From the result of the post extension motility of the rabbit semen at refrigerated temperature, at 24 hours post chilling, the forward progressive motility of QEY and CEY extended semen was significantly different from that of TEY extended semen. This experiment shows that at 24hrs post extension QEY and CEY extended semen have a good forward progressive motility i.e. the extenders are favourable for the survival of the sperm cells at refrigerated temperature (5°C).

At 48 hours, there was a rapid decline in the forward progressive motility of the extenders. QEY extender was relatively higher than the TEY and CEY extenders with 40%, 15% and 15% respectively. Progressive motility decreased as the period of storage increased. Comparatively, the progressive motility of the spermatozoa recorded after the first 24hours for the various extenders which was taken at 12hours interval for the next 120 hours varied. By 120 hours post storage at refrigerated temperature, all the sperm cells were found dead in EEY and TEY extenders except QEY extenders which showed 10% motility of the sperm cells, but are not viable again because the extenders was already contaminated with bacterial cells. Popoola *et al.,* (2017) reported that the superiority of some egg yolk sources (quail, chicken, turkey) has been attributed to the variable content of cholesterol, phospholipids and polyunsaturated fatty acids. In a cryopreservation experiment on Jackass semen, whole quail egg yolk was superior to whole chicken egg yolk in protecting sperm; attributed to its higher ratio of phosphatidylcholine and polyunsaturated fatty acids (Trimeche *et al.,* 1997; Burris & Webb, 2006; Popoola *et al.,* 2017). Tremeche *et al.,* (1997) in a cryopreservation experiment using donkey semen found out that quail egg yolk was superior to chicken egg yolk in protecting sperm, which was attributed to its higher ratio of phosphatidylcholine and polyunsaturated fatty acids (PUFAs). In another research carried out by Olurode & Ajala (2016), it was reported that the refrigerator temperature (5°C) had significantly higher motility values compared to room temperature (25° C) in all the trials. This could be attributed to the fact that refrigerator temperature help to reduce metabolic process in stored liquid semen which resulted in the utilization of nutrients such as fructose by the sperm cells (Aboagle & Terada, 2003). This observation is also in line with the reports of Bayemi *et al.,* (2010) and Udeh & Oghenesode, (2011) who found that refrigerator temperature recorded highest viability of stored bull and goat semen, respectively. However, addition of antibiotics could have prolonged the survival of spermatozoa at room temperature in line with the report of Foote & Bratton (1950) which indicated that extender containing antibiotic enhanced and prolong the usefulness of the semen. Although sodium citrate has been reported to be good extender (Adeyemo *et al.,* 2007; Udeh & Oghenesode, 2011), preserving sperm motility up to 24hours. The addition of either egg-yolk or goat milk or both prolonged the sperm viability in the extender beyond 24 hours due to composition of the added components (Olurode & Ajala, 2016).

The present results showed that the storage time at 5°C up to 48 hours significantly decreased the percentage of motile spermatozoa, irrespective of the extender used. In the same way, storage at 5°C up to 48 hours increased the percentage of dead spermatozoa. Interactions between the type of extender and storage time were not different on percentage of motile and dead spermatozoa. These results are in agreement with those of El-Gaafary (1994), Zeidan (1994) and El-Kelawy *et al.* (2012). Also, this trend was similar to the results obtained by Riad *et al.,* (2004). The observed reduction in semen quality when the duration of the storage increases may be due to the increase in lactic acid accumulation as a result of sperm anaerobic metabolism

leading to changes in both the osmotic pressure and pH of the media, which might exert a toxic effect on the sperm cells (Zeidan, 1994). Rabbit semen has been successfully stored at liquid state for short periods of time at 5°C (6 hours for fresh and 48 hours for refrigerated semen) without serious loss of its fertilizing ability (Daader & Seleem, 1997). The storage duration of extended rabbit semen, significantly decreased motility, spermatozoa viability and acrosomal damages, as well as the chemical characteristics (El-Kelawy *et al.,* 2012). However, experiments to evaluate their use in prolonging sperm viability and fertility throughout time are very limited. El-Gaafary (1994) using a Tris-yolk extender found that spermatozoa cooled and stored at 5°C for 24 hours had motility of 45% and after 48 hours dropped to 25%. This sharp decline in sperm viability throughout the preservation time might be due to chilling temperature.

The does synchronized with $PGF_{2\alpha}$ started showing signs of estrus 8 hours after synchronization. The signs of estrus includes; lordosis, tapping of the feet, moistened and oedematous vulva and mounting of each other. The luteolytic effect (regression of corpora lutea) of the $PGF_{2\alpha}$ prostaglandins (natural or synthetic) was used to induce and synchronize parturition or induce regression of the corpora lutea of pseudopregnant does (Theau-Clément, 2007). Dragan *et al.,* (1996) also reported when experimenting the use of prostaglandin analogues in rabbits that the product are well tolerated by rabbits, abortion and parturition are induced, sexual receptivity and fecundity are stimulated, the ovarian inactivity is cured, synchronization of kindling and the improvement of fecundity in does never pregnant before was achieved. Different authors have studied the efficacy of $PGF_{2\alpha}$ administered 2-3 days before insemination to synchronize the oestrus of does and the conclusions were diverse. When 200 mg of $PGF_{2\alpha}$ are injected 72 hours before insemination, Mollo *et al.* (2003) did not observe an increase of fertility. Theau-Clément (2007) also reported that one of the most likely hypotheses relies on the luteolytic effect of $PGF_{2\alpha}$ acting on pseudopregnant does. Thus, $PGF_{2\alpha}$ leads to the regression of existing corpora lutea and consequently, withdraws the inhibition of progesterone notably on oestrogen secretion, therefore allowing a new reproductive cycle. Thus, the prostaglandins may have an indirect action on the induction of receptivity, only on pseudopregnant does. There is little or no report on the use of $PGF_{2\alpha}$ analogue for synchronization for AI 12hours after injection.

Biostimulation before insemination should be easy to use, inexpensive, compatible with the animal welfare and well adapted to cyclic production. In this present study, all the females responded to the estrous synchronisation and/or ovulation induction treatments. Consequently, the 3 groups of rabbit does inseminated with the QEY, TEY, CEY extended semen gave a comparable successful fertilization rates. Pregnancy was confirmed by abdominal palpation 12 days after insemination on the experimental animals and 100% conception rate was recorded for all the groups.

CONCLUSION

The findings of this study reveal that QEY has a better protective ability to maintain motility of refrigerated extended spermatozoa of rabbit bucks. The improvement or decline in refrigerated post-chilled viability of spermatozoa with egg yolk of different poultry breeds in the refrigerated extender in this study could be attributed to the differences in the biochemical composition of the egg yolks.

RECOMMENDATION

It is recommended that rabbit buck semen extended with quail egg yolk at refrigerated temperature can be used as an extender in rabbitries using artificial insemination. Further studies are suggested to confirm the fertility success rate obtained in this study.

CONFLICTS OF INTEREST

Authors declare no conflict of interest.

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