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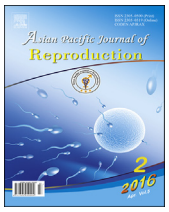

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The characterisation and cryopreservation of Venda chicken semen

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ABSTRACT

Objective: To characterize Venda cocks semen, find a suitable short-term diluted semen storage temperature, find a suitable cryopreservation cryoprotectant and to investigate cryoprotectant toxicity.

Methods: Semen was collected from six Venda cocks and evaluated macroscopically for semen volume, pH and sperm concentration. Microscopic sperm characteristics examined included were total motility (rapid, medium and slow) progressive and non-progressive motility. Velocity characteristics included curvilinear and straight-line velocity, average path velocity, linearity, straightness, wobble, amplitude of lateral head displacement and beat cross frequency.

Results: Results showed that the average semen volume was 0.3 ± 0.1 mL, the pH 6.9 ± 0.4 and the sperm concentration (6.8 ± 79.8) × 10^9/mL. A positive correlation was observed between body weight and semen volume (r = 0.38). Similarly a significant difference between the initial sperm total motility (TM%) of 87.5 ± 8.6 and samples stored for 24 h at 5 °C (55.0 ± 8.0) and 25 °C (30.6 ± 6.1) was recorded. The percentage live and normal sperm was 87.0% and 93.5% ($P < 0.05$) respectively. The TM% recorded was significantly different in samples supplemented with DMSO (46.0 ± 8.3), ethylene glycol (EG) (45.0 ± 12.2) and propanediol (PND) (21.8 ± 10.4), following thawing. Detailed velocity values showed consistent differences between the raw and cryoprotectant-free semen samples.

Conclusions: In conclusion, the Venda cock semen was subsequently found to have a higher TM% when stored *in vitro* at 5 °C. DMSO and EG were found to be suitable for the cryopreservation of Venda cock semen.

1. Introduction

The cryopreservation of cock semen has been extensively researched and sperm banking provides a possible effective method of maintaining superior male genetic material. However, cryopreserved cock semen has a limited on-farm use, due to its presumably low sperm motility with the primary role of the sperm being to fertilize the ovum [1]. An efficient method for chicken semen storage is thus necessary for future use. Generally, cold semen storage is used to reduce the metabolism of the sperm cell and to maintain sperm viability over an extended period of time [2]. These semen extenders and holding temperature play a significant role in maintaining cock sperm motility [3]. So it was found that in contrast to semen samples stored at 5, 15 or 25 °C [4], sperm stored at 41 °C showed a significant greater rate of sperm death [5]. Diluted semen stored at 2–5 °C retained its fertilizing capacity, even after 24 h [6]. The fertility rate following artificial insemination with frozen sperm is however low, than in fresh cock semen. So for example, hens inseminated with frozen sperm produced fewer fertile eggs, than those inseminated with fresh raw semen [7,8]. Methodologies for improved *in vitro* semen storage and thawing are thus required.

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The Venda chicken has not been extensively researched on *ex situ* conservation. In the past, information on the characterization of the breed was collected at the Agricultural Research Council (ARC) and there was very limited research on the cryopreservation of sperm for use in the chicken breeding programmes. According to the ARC, the Venda chicken breed was first recognised in the Venda region of the South African province of Limpopo. The breed is dual purpose, moderately large and multi-coloured with white, black and red as the predominant colours [9]. These Venda chickens are then known to survive under harsh conditions [10] and to have a low reproductive potential [11]. This breed reaches sexual maturity at 20 weeks of age, the hens are broody with good mothering ability and lay tinted eggs of a medium size. There is however cause for concern as the fertility of the experimental flock of Venda chickens at the ARC has declined over time. These indigenous chickens are generally raised by small scale farmers, with few resources in African countries mainly because of their hardness [12]. At present chicken genetic resources can only be conserved by maintaining a living flock which might be costly [9]. As a result, the cryopreservation of cock semen could play an important role in chicken breeding and genetic resource conservation.

Glycerol was initially used as the main cryoprotectant for the preservation of the sperm in most animals, including the stallion [13], ram [14] and the fowl [15]. However, cryopreservation was found to hamper fertilization in poultry [16]. The cause of this lowered fertility in artificially inseminated, cryopreserved sperm using glycerol as cryoprotectant was not identified, but may possibly be related to the osmotic shock following the rapid loss of glycerol from the sperm cell in the hen's reproductive tract [17].

Other cryoprotectants used for poultry semen have been dimethyl acetamide and DMSO [18,19]. These were then selected in terms of their low molecular weight and toxicity when used at low temperatures [20]. The addition of these cryoprotectants to cock semen resulted in an exposure to osmotic stress to sperm due to the osmotic efflux of extracellular water with a subsequent increase in cell volume as the cryoprotectant permeated and water concomitantly re-entered the sperm cell [21]. Further, ethylene glycol (EG) has a lower molecular weight (62.07 g/molar) and a greater membrane permeability than e.g. propanediol (PND) (76.10 g/molar) and DMSO (78.13 g/molar) [22]. It is suspected that EG permeates the sperm plasma membrane faster than PND and DMSO, hence causing damage to the sperm during equilibration and cryopreservation [23].

The semen analyses essential for the study of cock fertility generally includes the evaluation of sperm concentration, motility, morphology and semen volume [24]. The sperm motility analysis is repeatable and may then be linked to fertility [25]. This analysis generally makes use of manual and microscopic techniques. Alternatively a computer-aided sperm analyser (CASA) system objectively analyses the characteristics of sperm, providing scientists and breeders with a fertility prediction of individual cocks [26]. The aims of the study were to characterize Venda cocks semen and find the most suitable short-term diluted semen storage temperature and find a suitable cryoprotectant. In addition, the study was designed to determine the effect of cryoprotectant toxicity on sperm motility and the cryotolerance of sperm in individual Venda cocks.

2. Materials and methods

2.1. Experimental cocks

The flock consisted of pure-bred Venda cocks hatched from parent stock and housed at the Poultry Breeding Section of the ARC at Irene, South Africa. The cocks were vaccinated with live vaccines against Marek's disease, infectious bronchitis and Newcastle Disease at hatching. At week 24 of age, cocks were transferred to individual battery cages and fed a commercial diet *ad libitum*, until attaining a live body weight of 2.3 kg. All cocks were exposed to 16 h of light between 05: 00 and 21:00.

2.2. Semen collection and characterization

Semen collection was based on the method as described by Burrows and Quinn [27] from cocks at 26 weeks of age. This abdominal massage technique was used to collect semen three times per week from six Venda cocks [28]. Individual ejaculates were collected and placed in a thermos flask containing water at a temperature between 38 and 40 °C [29]. The semen being transported to the laboratory within 5 min following collection. All the experimental cocks were cared for, according to the guidelines of the ARC Animal Production Institute ethics committee (Ref: APIEC08/06).

Semen volume (mL) was measured visually using a graduated collection tube and the pH with the aid of a calibrated pH meter (Hanna instruments®, Portugal). Sperm concentration was determined using a JENWAY® 6310 spectrophotometer. The wavelength being set at 650 nm. The addition of 3 mL of a 2.9% Sodium citrate solution (pH 7.0), together with 15 µL semen in a cuvette was utilized and the absorbance was then converted to sperm concentration. The formula used: (11.170 × Absorbance) – 90. This was recorded in sperm/mL ($\times 10^9$/mL) [30]. Following the sperm swim-up preparation (10 µL of raw semen was mixed with 500 µL of Kobidil$^+$ extender, at 38 °C), 5 µL of the semen diluted 1:50 with Kobidil$^+$ extender, was placed on a warm microscope slide and covered with a warmed coverslip. This was then examined on a microscope fitted with a warm-plate (Omron®), at 37 °C. The TM% of the sperm was determined with clarity a Sperm Class Analyzer® (Microptic, Spain), at a magnification of ×10 (Nikon®, China). The total sperm motility was further classified into rapid, medium or slow motility and progressive and non-progressive motility. The sperm velocity characteristics measured included the curvilinear, straight line and average path velocity, linearity, straightness, wobble, amplitude of lateral head displacement and beat cross frequency.

Sperm morphology was microscopically evaluated by assessing 100 sperm per ejaculate per replicate. The 7 µL semen was mixed with 20 µL of an eosin/nigrosin stain, in a 0.6 mL graduated micro-centrifuge tube (Simport, Canada) [31]. Thereafter, 5 µL of the stained, raw semen sample was placed at the end of a microscope slide, smeared and fixed by air drying at 25 °C for 10 min, before evaluation [32]. Five µL of the stained, raw semen sample were then placed on the end of a microscope slide, smeared and fixed by air drying at 25 °C for 10 min before evaluation [33]. The sperm morphology was evaluated under a fluorescent microscope (BX51 TF®, Olympus, Japan). Viable sperm remained unstained and dead cells were totally or partially pink to red/brown. Viable sperm were further classified as morphologically normal or abnormal,