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EFFECTS OF REVIVE® ON SPERMIOGRAM OF DOG IN THE PRESENCE OR ABSENCE OF TEASER BITCH

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ABSTRACT

There are numerous indications for collection of semen from a male dog, yet there are various limitations that have been identified with the most common method used. Two adult male and one female local dogs with mean weight of 13.4 ± 0.7 kg were used in this study. Treatment orders (T_1 – T_4) used were 10 ml placebo administered orally 30 minutes before semen collection (SC) in the presence of teaser bitch (T_1); 400 mg of Revive® capsules administered orally 30 minutes before SC in the presence of teaser bitch (T_2); 10 ml of placebo administered orally 30 minutes before SC in the absence of teaser bitch (T_3) and 400 mg of Revive® capsule administered orally 30 minutes before SC in the absence of teaser bitch (T_4). A cross over design was used in this study in which each dog acted as his own control and participated in all the treatment groups. T_1– T_4 were assigned based on complete block design wherein each dog received each treatment thrice in a week. Each dog was ejaculated using digital manipulation method. The length of time to obtain ejaculate (Collection time, CT) was recorded. Semen volume (V), Sperm concentration (C), motility (M), and percentage live sperm (L) were determined for each ejaculate, using standard methods. The results were statistically evaluated using complete block analysis of variance (ANOVA) at level of significance of P=0.05. The mean collection time was 232.5±10.2secs for all treatments, CT was lower in T_1 compared to T_2 but the difference between dogs for CT was not significant. Mean Semen volume showed statistical difference between dogs. Mean values of V, C, M and L varied between the different groups. Results of this study showed that Revive® appeared not to have an effect on any of the ejaculate characteristics.

Key words: Ejaculate; Placebo; Preliminary study; Revive®; Semen; Sperm

INTRODUCTION

The indications for collecting semen from a male dog include artificial insemination, cryopreservation or diagnostic purposes and the most common method used for collection is digital stimulation (Mason, 2018). Under ideal conditions, this procedure is performed in the presence of an estrous bitch (Concannon, 2011). Although some dogs consistently give high quality semen samples when collected for semen, other dogs are more difficult to collect and give ejaculates with low numbers of sperm and other sub-optimal semen characteristics (Mason, 2018). This necessitates the use of sexual preparation in conjunction with se-

men collection. Sperm output from males of several species can be increased by supplementary sexual preparation (SSP). The use of SSP in conjunction with semen collection has been shown to optimize the number of spermatozoa in the ejaculate of rabbit, bull and stallions (Perumal, 2015). In dogs, the use of bitch in oestrus is a major method of sexual preparation used or the use of pheromones in the form of vaginal discharge from a bitch in estrus preserved on bedding or other absorbent materials (Kolster, 2018). Unfortunately, availability of teaser bitches and pheromones is frequently limited due to their monoestrous nature and clinicians are therefore forced to collect semen from dogs without sexual preparation (Kutzler, 2018). Although semen can be collected consistently from some dogs without the presence of a teaser bitch, collections done without availability of a bitch in oestrus often result into decreased semen volume and sperm count (Barber *et al.,* 2018).

Numerous pharmacologic agents have been shown to enhance male reproductive performance in many species (Kolster, 2018). Prostaglandin F2alpha ($PGF_{2}\alpha$) and Oxytocin are smooth muscle contracting drugs that have been used. $PGF_{2}\alpha$ increased the total number of spermatozoa in the ejaculate of bulls, rabbits, rams and stallions (Ungerfeld et al., 2018). Oxytocin is recognized as having endocrine and paracrine role in male reproduction. During ejaculation, a burst of oxytocin is released from the neurohypophysis into and stimulate contraction of the reproductive tract thereby aiding sperm release (Ungerfeld et al., 2016).

Revive® is a Chinese herbal product that boosts sexual performance in men. It enhances the relaxation of the corpora cavernosa and delays the lengthen period of the tunica albuginea, thereby increasing libido and sustaining erection firm enough for sexual satisfaction. Scientifically, research has proven that this product boosts the excitability of impulses from the brain and local nerves, hence allowing blood to flow in and fill the spaces within the tissues (Kedi Healthcare, 2018). The major active ingredient in Revive® is *Radix ginseng* and other herbs like *Epimedii, Fructus tribuli, Radix polygon multiflori, Cortex eucommiae, Cordyceps militaris* (Gao et al., 2020). Ginseng extracts improved sperm production in men and may have some usefulness in treating impotence. The ginsenosides, which appear to be the active components are thought to depress blood prolactin levels, thereby increasing libido. In one clinical study, 90 patients with erectile dysfunction were treated with ginseng saponins (600 mg orally per day). Treatment improved rigidity, tumescence, and libido, but not the frequency of coitus. (Bagchi et al., 2011; Lyttleton, 2013).

An increase in number of spermatozoa ejaculated in the dog would be of benefit when collecting semen for immediate insemination or storage. Historical work has documented an increase in the number of spermatozoa in the canine ejaculate if collection occurred in the presence of an estrous teaser bitch. However, collections done without the availability of a bitch in estrus often have decreased volumes and total number of spermatozoa (Valerie, 2009). Unfortunately, the availability of teaser bitches is frequently limited due to their monoestrous nature. There is need for a suitable pharmacologic agent as sexual preparation during collection, that could be able to increase the number of spermatozoa in the ejaculate with or without the presence of an estrous teaser bitch.

This study was carried out to determine whether the presence of an estrous teaser bitch will improve the ejaculate characteristics of dog to the same extent as administration of Revive® and whether the effects would be additive. It also sought to evaluate the effects of Revive® on the semen volume, sperm concentration, motility and percentage live sperm in the presence of an oestrous teaser bitch and the effects of Revive® on the spermiogram in the absence of an oestrous teaser bitch.

MATERIALS AND METHODS

Research design

This study was carried out in the Theriogenology unit of the Department of Veterinary Public Health and Reproduction COLVET, FUNAAB between 21st October and 21st December 2019. A cross over design was used in this study in which each dog used stood as his own control and also participated in all the treatment groups. The dogs were treated with one of two treatments 30 mins before each collection either in the presence or absence of teaser bitch. Treatments were either sterile saline(placebo) (10ml orally) or Revive® (Revive capsules,400mg orally).

Drug

Revive® is in capsule form containing (*Epimedii*(80mg)*, Radix ginseng*(80mg) *Fructus tribuli*(80)*, Radix polygoni multiflora* (80mg)*, Cortex eucommiae*(40mg)*, Cordyceps militaris* (40mg)). It is supplied as 30 capsules/ bottle (Kedihealthcare, 2018). The dosage of the drug used in this study was based on the dosage recommended by the manufacturer for humans

Animals

Three adult local comprising of 2 intact males and 1 intact pubertal, non-pregnant female with mean weight of 13.4 ± 0.7 kg, and age ranging between two and three years were used in this study. They were purchased from different owners who used them either for security or kept them as pet. The dogs were housed individually in concrete-floored kennels at the Veterinary Teaching Hospital, FUNAAB. They were fed once daily on household food (Spaghetti, Rice and noodles) supplemented with sufficient amount of proteins (fish) and palm oil, while water was provided *ad libitum*. They were dewormed with subcutaneous injection of 7.5% levamisole hydrochloride (Levacide®, Norbrook laboratories, Northamptonshire, United Kingdom) at the dose of 10 mg/kg, while external parasites were treated by dipping in Diazintol® (Animal Care, Nigeria) solution. Prior to onset of the study, the dogs were acclimated for 14 days in the animal containment facilities. During the acclimation period, the female dog was treated with Misoprostol (Cytotec®) by administering 200 mcg per os for one week during which vaginal smears were collected from her to stage her cycle. Thereafter, the male dogs were trained for two weeks to get them accustomed to semen collection by digital manipulation method. Two dogs that responded to training by maintaining penile erection and ejaculating were eventually used for this study (one of the male dogs though maintained penile erection but did not ejaculate). Semen was manually collected from the dogs twice weekly. Before commencement of the study, the dogs were adjudged to be clinically healthy and free of any reproductive disorders based on results of physical examinations, complete blood counts and fecal examinations.

Research procedure

Semen collection

Ethical approval for this study was obtained from the Research Ethics Committee, College of Veterinary Medicine (FUNNAB/COLVET/CREC/2019/10/04), Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The treatments given to the dogs were either sterile saline (10 ml orally) or Revive® (400 mg orally) The dogs received one of two treatments 30minutes before each collection either in the presence or absence of teaser bitch. The treatments were assigned based on complete block design such that the 2 dogs received all treatments. Four different treatment orders were designed (to ensure that treatment orders were not repeated) as stated below

- First treatment (T1) - 10 ml of sterile distilled water (placebo) administered orally 30minutes before semen collection in the presence of teaser bitch.
- Second treatment (T2) - 400 mg of Revive® capsule dissolved in 10ml of sterile distilled water) and administered orally 30 minutes before semen collection in the presence of teaser bitch.
- Third treatment (T3) - 10 ml of sterile distilled water (placebo) was administered orally 30 minutes before semen collection in the absence of teaser bitch.
- Fourth treatment (T4) - 400 mg of Revive® capsule was dissolved in 10 ml of sterile distilled water and administered orally 30 minutes before semen collection in the absence of teaser bitch.

Each dog received each of the above treatments thrice in a week (every other day of the week) before semen collection making a total of 24 collections for the two dogs. The sterile saline injections allowed each dog to stood as his own control. A period of 7 days was observed in between control and treatment. Semen was collected from each dog using digital manipulation method and length of time to obtain ejaculate from each dog was noted. The penis was vigorously massaged through the prepuce at the level of bulbus glandis until a partial erection developed, the prepuce was quickly retracted caudally past the bulbus glandis and firm constant pressure was applied to the penis behind the bulbus glandis by squeezing the penis between index finger and thumb. Digital pressure was further applied behind the bulbus glandis which constricted the penile venous return and this resulted in development of full erection displayed by maximal pelvic thrusting followed by ejaculation. The first and second (sperm rich fraction) portions of the ejaculate were collected into a sterile transparent sample bottle which was removed when the beginning of the third fraction was observed as characterized by appearance of clear prostatic fluid. Each dog was monitored following collection until the penis regressed to its ventral position and covered by the prepuce.

Semen macroscopic evaluation

The collected ejaculate was immediately transferred to a graduated sterile test tube. The volume, colour and composition of the ejaculate was immediately observed and recorded. The length of time to obtain ejaculate was also recorded to adjudge ease of collection for each treatment.

Semen microscopic evaluation

Evaluation of semen was immediately carried out following collection. This included determination of sperm concentration, percentage of motile spermatozoa, percentage of live spermatozoa.

Sperm concentration

Concentration was determined using modi-

fied Neubaeur haemocytometer method. Sperm suspension was diluted with cold 2.9% Na Citrate buffer in varying dilutions depending on the ejaculate. Diluted semen was mounted on the haemocytometer under the coverslip by capillary action. The spermatozoa were thereafter left for five minutes to settle before counting under light microscope. The mean number of cells counted in five squares in each counting chamber was determined; this was multiplied by dilution factor used for each ejaculate depending on the viscosity which was 1 in 20 in this study. This was then divided by volume per square multiplied by number of squares counted to obtain sperm concentration in millions per ml, this value was multiplied by volume of each ejaculate to obtain sperm concentration per ejaculate.

Sperm motility

Motility was assessed immediately after collection by subjective visual examination under a light microscope at 40X magnification by placing a drop of 2.9% sodium citrate (Na Citrate) buffer on a warm glass slide followed by a drop of the ejaculate covered with a cover slip. Sperm motility was expressed as percentage of total motile cells (slowly, moderately and rapidly progressive spermatozoa in the ejaculate) Albrizio et al., 2013.

Sperm viability

A thin smear was made from this preparation; one hundred spermatozoa were visualized in duplicate under light microscope at 1000X magnification for live spermatozoa. The average for each was calculated. Sperm viability was assessed using Eosin Nigrosin stain according to standard procedure. A drop of Eosin stain was placed on a warm glass slide in which a drop of semen was placed followed by three drops of Nigrosin stain.

Statistical Analysis

The results are given in terms of means and comparisons were statistically evaluated using complete block analysis of variance (ANOVA). Significant difference was acknowledged if $P \leq 0.05$. The hypothesis tested was that there was no change in the progressive motility of spermatozoa, but an increase in total number of spermatozoa, in the ejaculate, when dogs were treated with Revive® compared with placebo in the presence or absence of teaser bitch.

RESULTS

Following all the treatments T1(treatment with placebo in the presence of teaser bitch), T2 (treatment with Revive® in the presence of teaser bitch), T3 (treatment with placebo in the absence of teaser bitch) and T4 (treatment with Revive® in the absence of teaser bitch) all the dogs ejaculated and the ejaculation processes were without any complication.

Mean collection time was 232.5±100 seconds with a range of 211.2 to 253.8 seconds (Fig.1). The mean collection time for each dog showed no significant difference between T1, T3 and T4, but the mean difference between T1 was statistically longer than T2. However, the difference between dog for collection time was not significant (Fig.2).

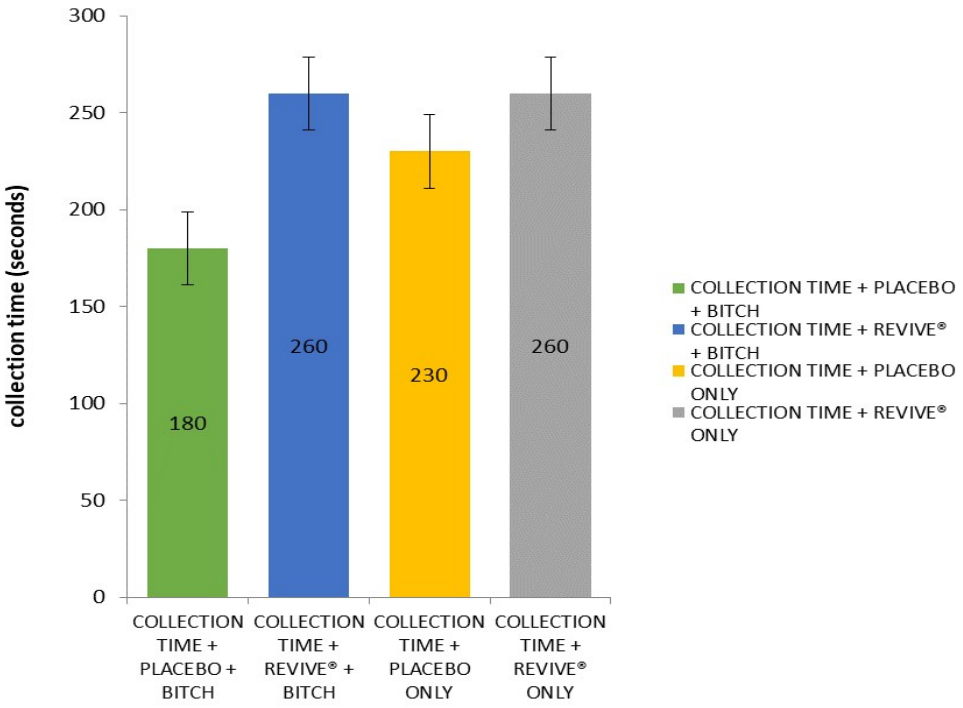

Figure 1: **Mean Collection Time of the Dogs**

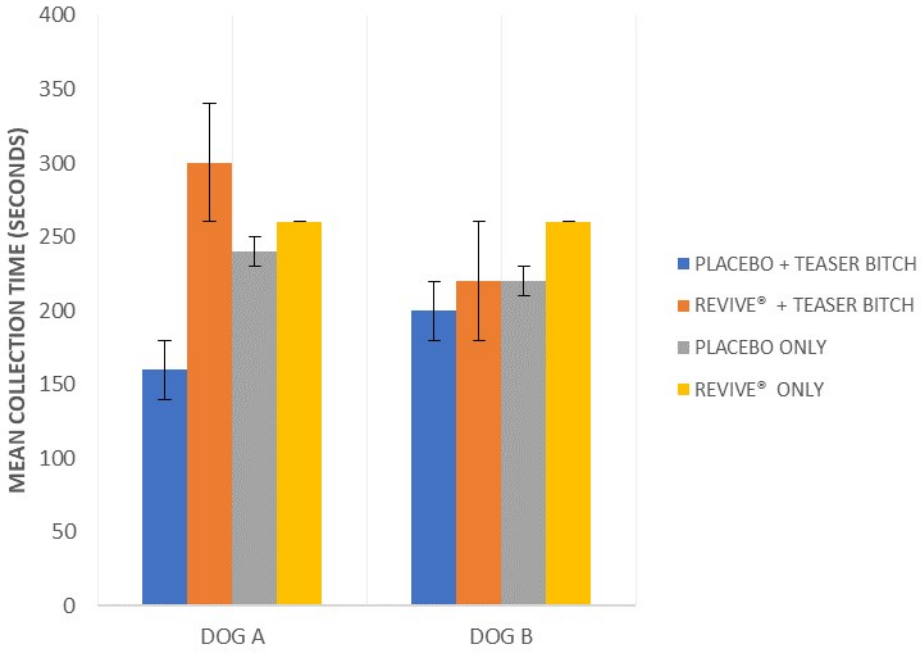

Fig 2: **Mean collection time for each dog**

The semen collected throughout the study was clear and did not have any debris or blood deposit. The colour of the sperm rich fraction (F2) was cloudy white, and this was the fraction that was evaluated within the first 10 minutes post-collection. Semen volume recorded for each dog showed no statistical difference between T2 and T4 compared to T1 and T3 but the difference between dogs was however significant (Fig.4). Difference between dogs was not significant (Fig.5) when measuring the mean sperm concentration in the ejaculate. There was variability in the concentration obtained for the different treatments (Fig.6) wherein T4 had the highest sperm concentration while T2 had the lowest value but they were not statistically significant.

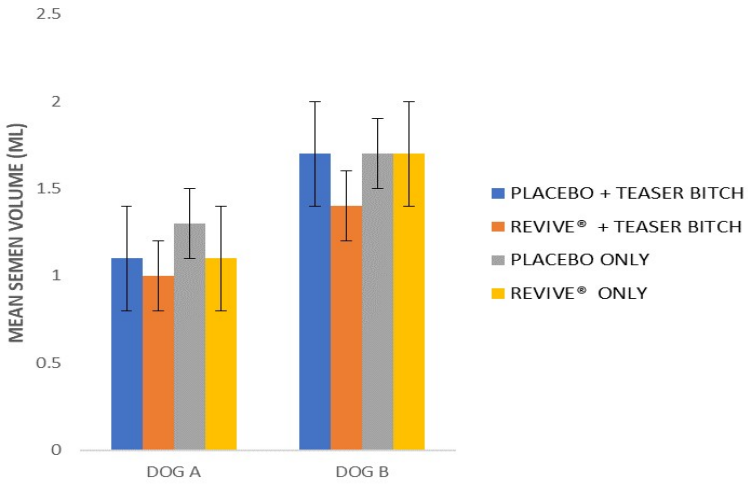

Fig.3. Mean semen volume of the dogs

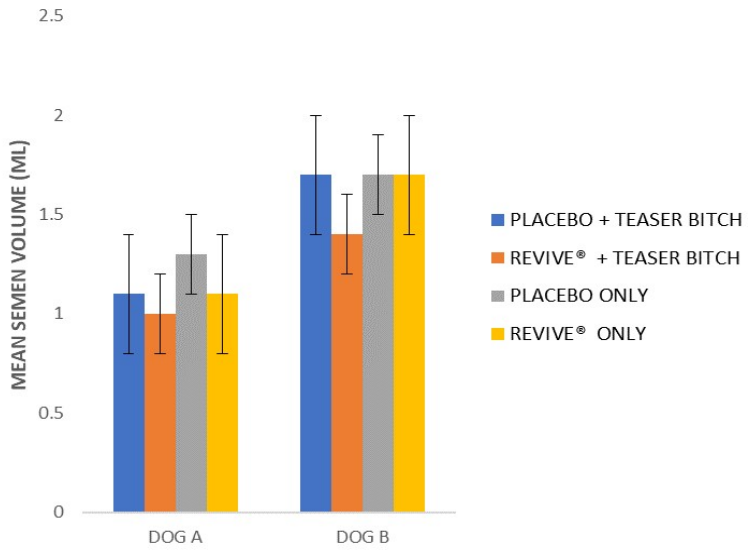

Figure 4. Mean semen volume of each dog

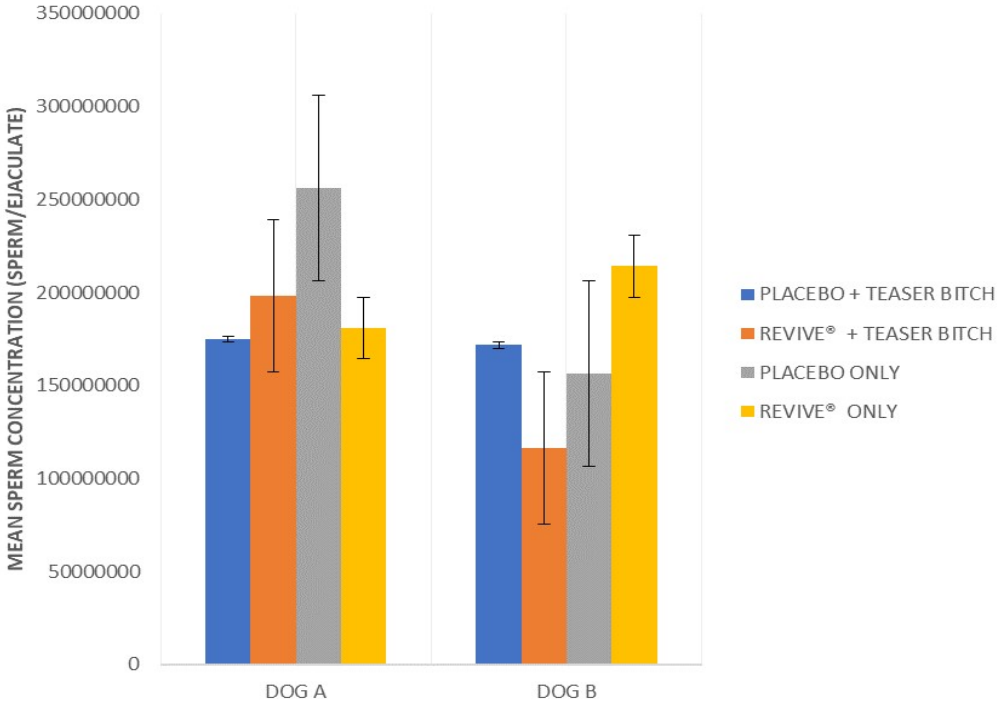

Fig.5. Mean sperm concentration of each dog

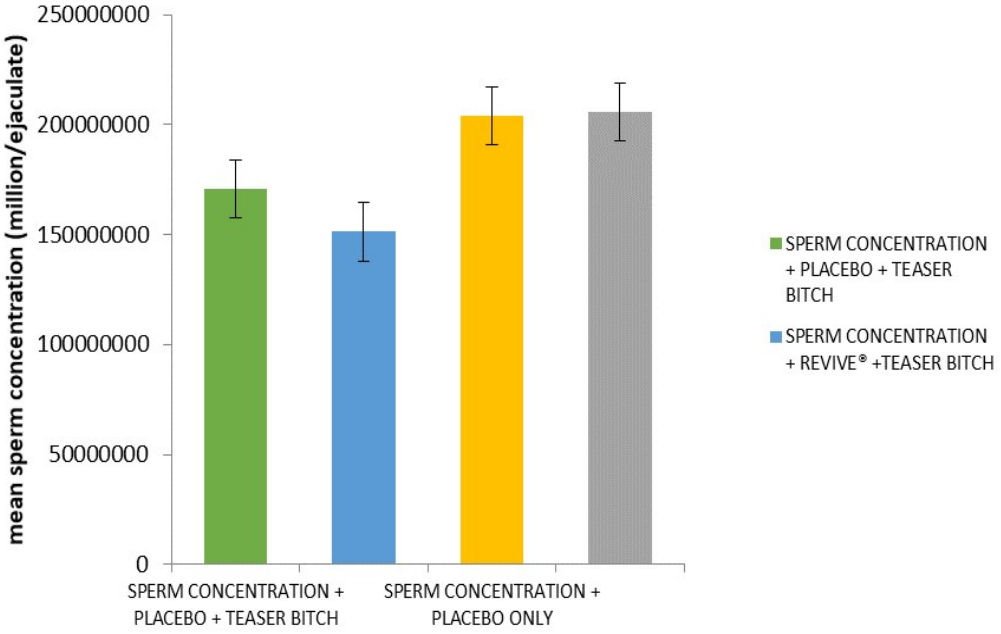

Fig.6. Mean sperm concentration of the dogs

The mean % live sperm was 95.3±0.8 % for all treatments in this study. (Fig.7). The treatments given did not make any significant difference on the % live sperm in the ejaculate of the dogs and the difference between dogs was also not significant (Fig.8). T2 had the highest sperm motility but there was no difference in the mean between treatments (Fig.9) and between the dogs (Fig.10).

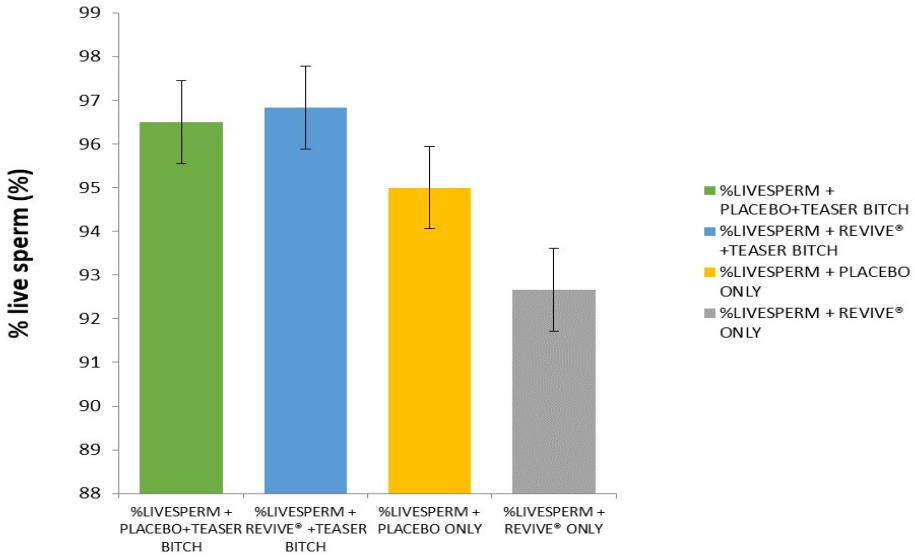

Figure 7. Mean % live sperm of the dogs

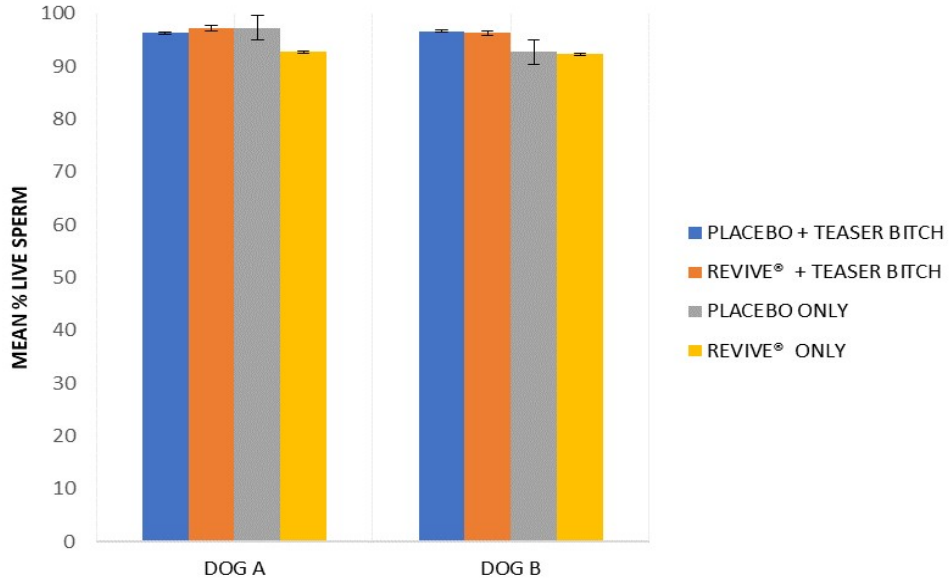

Figure 8. Mean % live sperm of each dog

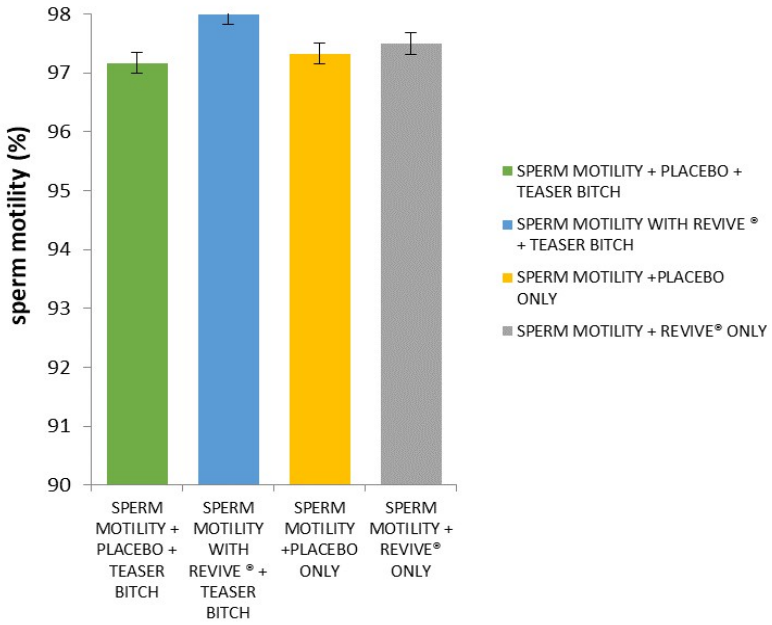

Figure 9. Mean sperm motility of the dogs

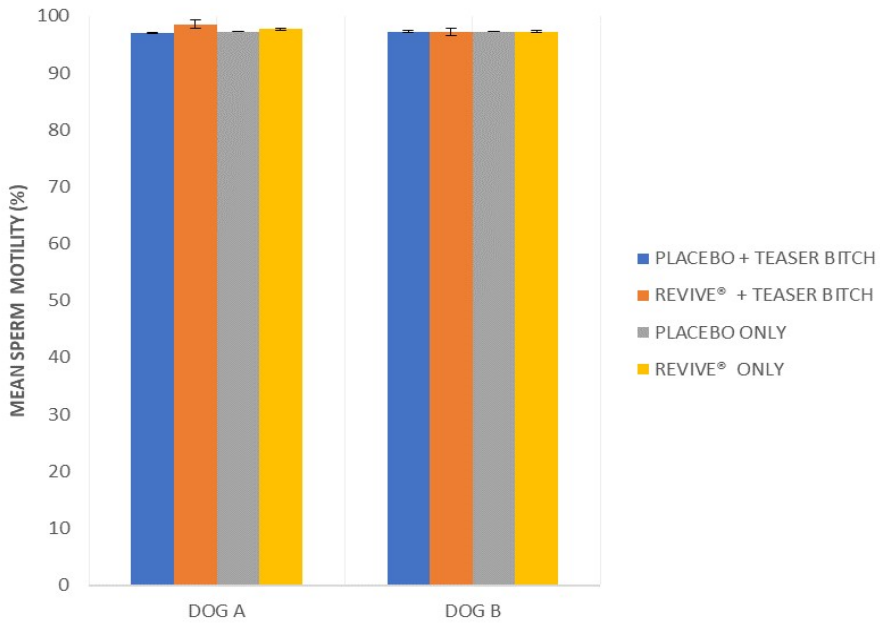

Fig.10. Mean sperm motility for each dog

DISCUSSION

Results of this study showed that Revive® appeared not to have an effect on any of the ejaculate characteristics of semen when used in the manner described in this study. Revive® is a herbal product known to boost sexual performance in men (by sustaining strong and hard erection). Its constituents are mainly made up of various anti-oxidants and natural herbs known to be used for aphrodisiacs. Its mechanism of action which is by acting on impulses from blood and local nerves thus allowing blood to flow in and fill the penile tissues (Kedi HealthCare, 2018) is thought to be similar to that of smooth muscle contracting drugs (e. g. oxytocin) that have previously been used for sexual preparations before semen collection (Ungerfeld et al., 2018). The collection time that was longer in dogs treated with Revive® before semen collection whether in the presence or absence of teaser bitch may be attributed to the fact that collection was done 30 minutes following administration of Revive®. It is possible the timing was too short for exhibition of its full effect. An alternative study design in which repeated measures of collection would be done following administration of Revive® may further explain this. Multiple obstacles exist that impede a controlled clinical trial evaluating effect of neutroceutical supplementation on semen parameters in dog (Lopate, 2010). One major obstacle is difficulty finding lengthy studies and observing dogs with suitable semen quality for evaluation in a comparative trial. This was one of the major obstacles encountered in this study in which the number of dogs acquired at the onset of the study could not be used all through because one was aspermic.

CONCLUSION

This present research was carried out as a preliminary study, further research is needed using more dogs and other techniques before the use of Revive® is considered ineffective as supplementary sexual preparation in dog semen collection.

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