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RESEARCH ARTICLE

MEAN SPERM CONCENTRATION AND PERCENT MOTILITY OF EXTENDED PORCINE SEMEN AS AFFECTED BY ANTIBIOTICS FROM SELECTED SOURCES AND STORAGE TIME

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ABSTRACT

Two crossbred boars were trained to mount a dummy sow and semen was collected through the gloved-hand technique. The semen was extended with Beltsville L1 (BL-1) extender containing penicillin-streptomycin antibiotics from 5 pharmaceutical sources namely: Sigma, Pfizer, Verticare, Squibb and Butler. Evaluation of semen after 1, 24 and 48 hours storage at 10^0C for sperm concentration and percent motility was used as a basis for determining the effect of ingredients in antibiotic from different sources and storage time on spermatozoa. Different sources of antibiotics used did not affect ($P>0.05$) sperm concentration and percent motility. However; storage time had effect on percent motile sperms.

INTRODUCTION

In an attempt to holistically crystalise what Artificial Insemination (A.I) truly is, the views and positions of several authorities were considered to arrive at this definition: Artificial Insemination (AI) is a reproductive technique employed for the harvesting of semen by means of appropriate instruments and the subsequent introduction of such semen either in its concentrated or diluted form into a physiologically receptive female (Hafez, 1987; Flowers, 1997; Althouse, 1997; Auroux *et al.*, 1991; Poolperm, 1997 and Egbunnike, 1994).

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A.I. in pigs was first performed in Russia by Ivanov at the start of the 20th century and later developed at Russian State farms in the 1930s (Gadea, 2003). True development and commercial application of AI in pig production did not take place until the 1980s in developed Countries (Grabo, 1990; Johnson *et al.,* 2000) when insemination protocols were standardized. Artificial insemination in swine is economically advantageous and simpler than natural service under the labour and management service of large, farms. A minimum of 2 billion live sperm cells in an adequate volume of fluid are required to obtain adequate conception rates (Selk, 2007). If 2 inseminations/animal is required, then 43 animals

can be inseminated with 1ml of semen. Therefore, from 10ml of ejaculate, 430 inseminations are possible.A.I is therefore an excellent means of judiciously using and conserving semen. In the tropics where there is a strong need to improve indigenous breeds, the many advantages of A.I. cannot be over emphasized. One of the biggest constraints to the use of A.I. however, is the lack of effective methods for preserving the fertilizing capacity of swine spermatozoa during storage and transportation (Anjer *et al.,* 2003; Just *et al.,* 2004; Kuczynski *et al.,* 2003; Ottolini *et al.,* 2006 and W.H.O, 2001; Adeleye and Omitogun, 2009). This is why the quality of extender used (among other factors like handling, storage temperature, whether storage is aerobic or anaerobic, microbial load, and so on) is of great importance.

A good extender must provide adequate nutrients for metabolism, protection against cold shock, buffering against pH shifts, proper Osmotic balance, volume for multiple inseminations and inhibition against Bacterial growth (Foote, 1974; Orok 1980; Otite, 2000). Many extenders have been used to extend stored semen, like whole milk (Ebertus, 1963), Cream gelatin (Hughes and Loy, 1970), evaporated milk glucose-glycerol (MacCall and Sorensen, 1971), egg yolk-sugar-glycerol (Krouse and Grove, 1967; Ostuda *et al.,* 1968; Nashikawa *et al*., 1968), mare's milk (Kamenev, 1955), skim milk-glucose (Rajamannan *et al.,* 1968) and tri-glucose-citric acid-egg yolk (Cranwell, 1970; Anderson, 1971). Otite (2000) also posited the use of *Hibiscus sabdarifa* (popularly known as 'zobo'in Nigeria) as an extender in canine semen because of its possession of a high calorific value, its high ascorbic acid content and its low fat content. During *in vitro* sperm preservation, a good diluent (extender) must also provide an environment that prevents lipid peroxide formation (Jones and Mann (1977a and 1977b). One other problem that has to be confronted head long in *in vitro* sperm preservation is the problem of cold shock. When ram, bull and boar spermatozoa are suddenly cooled to temperatures between 15^0C and O^0C, there is irreversible loss of motility and metabolic activity commonly called "cold shock". Other events that follow are leakage of intracellular proteins and enzymes, alteration of cellular cations, increased membrane permeability and a loosening of the acrosome of susceptible sperms (Hannock, 1952; Walton, 1957). Pursel *et al.* (1972a and b) have also underscored the importance of storage temperature and duration of storage on sperm quality. Another very important contributory factor to lower male fertility is the association of pathogenic bacteria in the male genito-urinary tract. In 1967, Dennis demonstrated immobilization obtained from *Vibrio fetus*, a common pathogen in ram and bulls. Orok (1980) reported that live pathogenic *Escherichia Coli* obtained from urinary and cervical cultures produce profound depression on motility and viability of human sperms *in vitro.*

Crouch *et al.* (1972) failed in their attempt to treat a stallion that was shedding *klebsiella aerogenes* in his semen; therefore it was necessary to treat the semen rather than the stallion for effective control. This may have been as a result of contamination during semen collection necessitating the use of antibiotics in semen (Akhter *et al., 2007*). The level and type of antibacterial agent, length of incubation, storage temperature and dilution ratio, are all important factors to be considered before extending semen with antibiotics (Althouse, 1997; Gopalkrishnan *et al.,* 1994; Teague *et al.*, 1971; Auroax *et al.,* 1991; Omitogun and Oyeleye, 2006; Adeleye and Omitogun, 2009). Concentration of semen per ml of fresh or extended semen and sperm motility are important because they are both positively correlated with conception rate (Hafez,1987; Auroux *et al.,*1991; Flowers,1992; Flowers,1997). Colenbrander and Kemp (1990) recommended a motility of 50%-90% in pigs. They posited that circular or reverse motions of spermatozoa were signs of cold shock; Oscillatory motions (signs of aged semen); No motion (dead spermatozoa) and progressive or rapid movements (signs of normal spermatozoa). This study was undertaken with the broad objective of determining if the ingredients in antibiotics have any significant effect on spermatozoa concentration and motility of extended semen over different storage periods.

MATERIAL AND METHODS

The experiment was conducted in the Tuskegee institute, Alabama, in the United States of America.

Experimental design

There were five treatments and five replications. The treatments were:

1: Semen + BL—1 + Crystalline Pen-Strep (from Sigma company)
2: Semen + BL—1 + Commercial Pen-Strep (from Pfizer Company)
3: Semen + BL—1 +Commercial pen-Strep (from Veticare Company)
4: Semen + BL—1 + Commercial Pen-Strep (from Squibb Company)
5. Semen + BL—1 + Commercial Pen-Strep (from Butler Company)

Preparation of extenders

BL—1 extender was prepared according to the formula of Pursel *et al.* (1972a) (Table 1). There were five flasks of BL-1 extender identified as flask #1 through #5. To flask #1 was added in crystalline form, one gram of dihydrostreptomycine sulphate and 1 x 10^6 I.U. penicillin-G; this was the control extender and was designated treatment #1.To flasks #2 through #5 were added 2.5 ml of commercial penicillin-streptomycin sulphate in aqueous suspension (4 x 10^4 I.U. procaine penicillin and 0.25 grams dihydrostreptomycin sulphate per ml) from selected sources. Each flask (#2-#5) received antibiotics from one of the four commercial sources. These were the experimental treatments and were designated treatment #2 through #5, respectively (see Experimental Design).

Table 1. Beltsvililles L1 (BL-1) composition

Ingredients	Amount*
Glucose (dextrose) anhydrous	29.0g
Sodium citrate dehydrate	10.0g
Sodium bicarbonate ($NaHCO_3$)	2.0g
Potassium chloride (KCl)	0.3g

*Dissolved and brought to 100 ml with distilled water.

Semen collection

Two crossbred boars, 18 months of age, were trained to mount a dummy sow. Semen was collected by the "gloved—hand" technique (Hancock and Howel, 1959) into a 250—ml insulated flask. The opening of the flask was covered with a milk—filter disc to separate the gel. Semen was visually fractionated into "sperm—rich" and sperm-poor" portions at collection, and only the sperm—rich portion was used. Semen from the two boars was pooled.

Semen processing and treatment allocation

Once in the laboratory, semen was held for about 30 minutes to equalize its temperature with the room temperature (22^0 to 24^0C). The concentration of the semen was determined using the hemacytometer (Pursel *et al*, 1972, a,b) Five 30—ml propylene bottles with screwed tops were obtained. Aliquots of the "sperm—rich" semen were extended with each of the treatments (#1 — #5) so that each 25 ml storage volume in each of the propylene bottles with screwed top contained 3 x 10^9 per ml. The five bottles were stored at 10^0C. Semen samples from the treatments were evaluated after 1, 24, and 48 hours storage for percent motility and concentration.

Evaluation procedure

All samples were coded before evaluation so antibiotic identity was not known during evaluation. At the end of storage time intervals, 2 ml samples were pipetted from the bottles after they had been shaken thoroughly. Percentage motility was estimated using 0.5 ml of the pipetted volume. The sample for motility estimate was held at 37^0C for 30 minutes. From the remaining volume, one ml was used in the determination of concentration.

For estimation of percentage motility, two drops of the semen sample which had been held for 37^0C for 30 minutes were placed on the center of two pre-warmed slides. The slides were covered with pre-warmed slide covers. The slides were observed under 10 x objective of the microscope and the percentage motility of the sperm cells was estimated from an average obtained from the observation of the two slides.

The concentration was determined using one ml of the sample. This volume was pipetted into a red blood cell pipette and placed on an electric shaker for 30 seconds, after which the first few drops of the sample were discarded through the capillary

end of the pipette by creating a positive pressure inside the pipette chamber. The capillary end of the pipette was wiped off, then a small drop of the sample was placed under the hemacytometer cover glass, which had been placed squarely over the counting chamber of the hemacytometer. This was repeated on the other chamber of the hemacytometer. The cells were allowed to settle for 3 to 5 minutes before slides were observed under 100-x objective of the microscope. Five squares on the hemacytometer chamber were counted, starting at the upper left corner, then the upper right—hand corner, the bottom right hand corner, the bottom left-hand corner, and finally moving three squares from the bottom left-hand corner to the right, then three squares up vertically, and counting the squares. All sperm cells with heads within the counting squares and those on the top and left boundary lines were counted. An average from the two counting chambers multiplied by 10^7 gave the concentration of the sample.

RESULTS AND DISCUSSION

Concentration (10^7): Evaluation of semen samples for concentration at hour-1, 24, and 48 post storage showed mean sperm concentration of 30.2, 30.8, and 31.2, respectively. These means were not statistically different (P> .05). Sperm fractions extended by the different antibiotic sources were not significantly different at 5% (p>0.05) level of probability. The mean concentration of sperm extended with antibiotics from Sigma, Pfizer, Veticare, Squibb, and Butler were 30.7, 30.8, 30.6, 30.9 and 30.8, respectively (Table 2).

Motility: Duration of storage appeared to influence percent motility of sperm in semen extended with antibiotics. The mean percentage motility decreased from 97.1 after a one-hour storage to 79.5 in 24 hours, and 71.8 in 48 hours. Means for hour-24 and 48 were not significantly different (P >05), but they were significantly different from hour-l (P <. 05). The mean percent motility of

Table 2. Mean sperm concentration (1 x 10^7) of extended fresh semen as affected by antibiotics from selected sources and storage time*

Antibiotic source	Storage Time Hr-1	Storage Time Hr-24	Storage Time Hr-48	Mean ± S.E.
Sigma	29.8	30.4	31.8	30.7 ± 0.4
Pfizer	30.4	30.8	31.2	30.8 ± 0.4
Veticare	29.4	31.0	31.4	30.6 ± 0.4
Squibb	31.2	30.6	31.0	30.9 ± 0.3
Butler	30.4	31.2	30.8	30.8 ± 0.4
Mean ± S.E.	30.2 ± 0.2	30.8 ± 0.4	31.2 ± 0.3	

*Each value based on 5 observations.

Table 3.Mean sperm motility (%) of extended fresh boar semen as affected by antibiotics from selected sources and storage time*

Antibiotic source	Storage Time Hr-1	Storage Time Hr-24	Storage Time Hr-48	Mean ± S.E.
Sigma	94.2^{b}	84.4^{a}	76.0^{a}	84.9 ± 2.1
Pfizer	98.0^{a}	85.0^{a}	74.0^{a}	85.7 ± 2.7
Veticare	97.4^{a}	79.0^{ab}	69.2^{ab}	81.9 ± 3.4
Squibb	98.4^{a}	72.0^{b}	66.0^{b}	78.8 ± 4.1
Butler	97.4^{a}	77.0^{ab}	74.0^{a}	82.8 ± 2.9
Mean ± S.E.[1]	97.1 ± 0.4^{a}	79.5 ± 1.4^{b}	71.8 ± 1.2b	

*Each value based on 5 observations.
[1]Any two means within a row/column with different superscript are significantly different (P < .05).

spermatozoa in semen samples extended with crystalline pen-strep from Sigma and pen-strep combinations from Pfizer, Veticare, Squibb, and Butler were 84.9, 85.7, 81.9, 78.8, and 82.8, respectively. These means were not significantly different (P > .05) (Table 3). Results of these experiments indicate that ingredients in the antibiotics had no significant effect on sperm motility. However, there was a difference of 18% in sperm motility between 1 and 24 hours storage period as compared to a difference of 8% between 24 and 48 hours storage period. There was reduction in motility between the observation at 1 and, 24-hour in the sample containing antibiotics from Butler, Veticare, and Squibb as compared to that observed for Sigma and Pfizer. The average reduction in motility was 21,7% for Butler, Veticare and Squibb, whereas the average for Sigma and Pfizer group was 11.4%. Between hour-24 and hour-48, Pfizer and Veticare average reduction was 10.5%, whereas Butler had only 3% reduction in motility. This indicates that storage time influences the percentage of motile sperm in semen extended with antibiotics.

Conclusion and Recommendatios

Storage time is an important factor in determining sperm quality as sperm concentration and percent motility progressively decreased with increase in storage time. The different brands of antibiotics had different effects on motility, which reproductive physiologists may want to pay closer attention to. For instance, while average reduction in motility with Butler, Verticare and Squibb was 21.7%,it was just 11.4% with Sigma and Pfizer. But it should be noted that between 24hrs-48hrs, reduction in, motility for Pfizer and Veticare was 10.5% whereas it was only 3% in Butler. It is advisable that where possible, stored semen should be used for insemination within the first 1-3 days of storage. It is also advisable that inseminations be done with semen from the different Treatments to determine conception rates so that categorical assertions about the fertilising abilities of the sperms from the different Treatments can be made.

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