FERTILITY RESPONSE OF ARTIFICIALLY INSEMINATED BROILER BREEDER HENS TO SEMEN DOSE, INSEMINATION FREQUENCY AND SEMEN DILUTION

BY

Abosede Olabisi BOLARINWA
Matriculation Number: 126346

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CERTIFICATION

I certify that this study was carried out by Abosede Olabisi BOLARINWA with Matriculation Number 126346, in the area of Animal Reproductive Physiology and Artificial insemination, Animal Physiology and Bioclimatology Unit, Department of Animal Science, University of Ibadan, Ibadan, under my supervision.

Supervisor

Dr E. O. Ewuola

B. Agric. Tech. (FUTA), M.Sc., Ph.D. (Ibadan), RAS

Animal Physiology and Reproduction,

Department of Animal Science,

University of Ibadan,

Ibadan, Nigeria.

DEDICATION

I dedicate this thesis to God Almighty for the grace and favour given to me in achieving this breakthrough, also to my wonderful parents and husband for their maximum support during the course of this programme.

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ABSTRACT

High ratio of cock to hen required for optimal fertility under natural mating usually leads to increase cost of producing day old chicks. Adoption of Artificial Insemination (AI) could maximise the use of cocks thus reducing production cost. However, required semen dose and frequency of insemination for fertility in breeder chickens have not been documented in Nigeria. Therefore, effects of semen dose, frequency of insemination and semen dilution on egg fertility in broiler breeder chickens were investigated.

Marshall broiler breeder hens (n=348, 32 weeks, 2.80±0.34 kg) and 15 cocks (32 weeks, 3.50±0.45 kg) were used. One hundred and twenty hens were randomly divided into five treatments. Treatments one to four were inseminated with 0.02 (containing $5x10^6$ motile spermatozoa, T1), 0.04 (T2), 0.06 (T3) and 0.08 mL (T4) of pooled raw semen harvested from cocks using established technique, while treatment five (T5) hens were mated naturally. Both processes were done for two successive days. Eggs were collected daily and incubated weekly for four weeks to determine fertility and embryo mortality. Four groups of 25 hens each, were inseminated (0.02mL/hen) with undiluted semen at three (D_3), six (D_6), nine (D_9) and twelve days (D_{12}) intervals. Fertility and hatchability were assessed. Another batch of semen were collected and divided into four parts: Undiluted Semen (US), others were diluted at 1:1 with Modified Ringer's Solution (MRS); Normal Saline (NS) or 1% Dextrose Saline (DS). Spermatozoa motility was measured hourly until it dropped to 50% at 27.96±0.38°C. The treatments were inseminated into 80 hens to determine fertility. In a 3x3 factorial arrangement, another 81 hens were divided into nine groups and inseminated with semen diluted with DS at 1:0 ($T_{1:0}$), 1:1 ($T_{1:1}$) and 1:2 ($T_{1:2}$) each at three, six and nine days intervals to assess fertility and hatchability. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$.

In the first week, fertility in T1 (94.3±1.9%), T2 (94.5±3.2%), T3 (95.2±1.9%) and T4 (97.1±2.9%) were significantly higher than T5 (76.9±3.0%). Embryo mortality was similar among treatments. Fertility up till 7 days in inseminated hens was above 90%, while at days 8 and 9 it was 87.0±4.7% and 74.2±9.3%, respectively. However, there was a decline from day 10 (69.3±9.3%) to day 22 (0.0±0.0%). Fertility in D_3 (80.4±1.8%), D_6 (74.3±1.8%) and D_9 (76.3±1.5%) were significantly higher than D_{12} (67.8±2.2%), while hatchability was similar. At third hour, spermatozoa motility was higher in US (71.7±I.7%) and NS (71.5±1.7%) than MRS (61.7±1.7%) and DS (58.3±4.4%), while fertility was highest in DS diluent. Fertility among dilution ratios were similar at 3-day interval but was lower in $T_{1:2}$ (62.4±7.2%) and $T_{1:1}$ (50.7±10.8%) than $T_{I:0}$ (75.8±8.2%) at 6-day interval. At 9-day interval, $T_{1:2}$ (59.95±1.8%) and $T_{I:1}$ (66.9±6.1%) were lower than $T_{1:0}$ (80.70±6.0%), while hatchability was similar.

Artificial insemination improved egg fertility. Insemination frequency up to 9 days and semen dilution of 1:2 at 3 days intervals sustained fertility and hatchability in broiler breeder hens. Normal and dextrose saline are suitable as cock semen diluents.

Keywords: Cock semen dilution, Egg fertility, Embryo mortality, Insemination dosage

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CHAPTER ONE

INTRODUCTION

1.1 Background of the study

There has been a steady increase in the production of broiler breeders due to the increase in the demand of day old broiler chicks. This can be partly attributed to the ban in the importation of frozen chicken and turkey into the country. Breeder farmers are interested in well adapted strains which can adapt to local environment for producing quality hatching eggs (Hossain *et al*., 2005).

Mating system in poultry breeder production is an important tool that determines the success of any breeding programme, if properly harnessed. Low reproductive performance attributed to low fertility and hatchability has been the major problem of natural mating in Nigeria poultry industry (Udeh *et al*., 2011). Larger percentage of poultry farmers that raise parent stocks, practice natural mating, which is predominant in the tropical region. There are attributed problems associated with natural mating system which makes artificial insemination an option for breeding programme. Transfer of diseases between the male and the female during mating as a result of skin contact can be prevented using artificial insemination. Natural mating can be stressful on hens especially due to the heavy weight of the cock, which can lead to reduced fertility as a result of unsuccessful copulations (Attia *et al*., 1993).

Also, aggressive behaviour during natural mating, may cause injuries to the hen and lead to reduced fertility potential. Semen quality and quantity cannot be monitored. A cock deposit much more semen during a single ejaculation than what is needed to fertilize an egg, which lead to wastage of sperm cells and economic loss. The number of cock to hen ratio in natural mating leads to increase in cost of feeding and space needed to keep the cock. Other factors known to affect fertility include leg disorders,

overweight, a reduction in the biological value of eggs and the ability of oviducts of older female to accept sperm (Lukaszewicz, 2001).
Application of artificial insemination is common in the poultry industry in developed countries (Surai and Wishart, 1996). However in Nigeria, measures has not been put in place in the industry to regulate artificial insemination procedure as a result of lack of insemination schedule on dose of semen, rate of insemination and dilution ratio of semen require for optimal fertility. Artificial insemination technique in poultry lengthen insemination frequency with lesser semen dose of insemination (Froman, 2011). Artificial insemination in poultry has relatively better fertility compared with natural mating, a technique adopted for genetic improvement (Gill *et al.*, 1999). Production of hatchable eggs as a result of reduced libido is an economic problem in which artificial insemination has solved. Artificial insemination increases the efficiency of male usage by increasing the mating ratio (Habibullah *et al.*, 2015), and using appropriate diluent to improve the quality of the semen (Adebisi and Ewuola, 2019b).

Artificial insemination also helps resolve preferential mating which could impair fertility. Fertile eggs laid in a cage are no longer issue and the use of cages, system with several hens to one male, which usually reduce fertility rate has been eliminated (Surai and Wishart, 1996). Artificial insemination reduces the transfer of venereal disease as caused by skin contact among male and female animals (Chaudhury, 1996). The process of carrying out AI is under hygienic condition which allows for screening of semen and checking for the quantity and quality in order to prevent problems associated with male infertility. Artificial insemination increase production efficiency, reduce semen wastage and also reduces cost of feeding cocks, which can increase the profit margin of the farmer. Application of artificial insemination can increase fertility by an additional 5 - 10%, even with previous fertility levels of between 80 - 85% (Gee *et al.*, 2004). This also helps to enhance the rate at which genetic improvements are made. On the other hand, a few demerits accompany AI. This include inadquate skilled labour and logistics of semen transportation. In addition, stress disrupts successful implementation of AI in the commercial poultry production (Reiber and Conner, 1995).

Artificial insemination in Nigeria has gained little recognition among farmers compared to natural mating. In poultry, AI has not received extensive application, some of the challenges involve in participation, adaptation and utilisation of AI in Nigeria include difficulties in obtaining necessary equipment, laboratory supplies and professional personnel. This contributing to the low rate of AI development.

In the past, most of the researches that have been done on AI in poultry in Nigeria Ewuola *et al.* (2020) reported optimal fertile period of 5 days and insemination frequency of 3 days using 0.02mL semen dose in Nera Black layer chicken. Better fertility and hatch parameters was reported in Shika Brown breeder chicken hens when insemination was done in the morning in the Northern grassland region of Nigeria while higher fertility was reported using undiluted semen inseminated twice a week Obidi *et al.* (2008). Adebisi and Ewuola (2019a) reported high fertility in indigenous turkey hens up to three weeks after insemination and recommend insemination frequency of one to two weeks with 0.02mL semen dose. However, there is dearth of information on insemination schedule and oviductal sperm storage effect on egg fertility in broiler breeder hens in Nigeria. Despite the success recorded in trials involving AI in Nigeria, there is still indiscriminate use of semen dose to breeder hens in poultry breeding programmes, thus leading to semen wastage. Also, there has not been proper documentation on insemination schedule particularly on least viable semen dose and frequency of insemination for optimum fertility under Nigerian conditions. Therefore, this study was aimed at investigating semen dosage, oviductal sperm storage, frequency of insemination and semen dilution for optimal fertility in broiler breeder hens.

1.2 Study Objectives

1.2.1 Main Objective

The main objective of this study is to create an artificial insemination protocol necessary for optimum fertility and hatchability in broiler breeder hens for poultry industry in Nigeria.

1.2.2 Specific Objectives

Precise goals of the work are to:

i. evaluate the storage capacity of broiler breeder hens using the fertile period length.

ii. examine the influence of semen dose on fertility, death of embryo and hatching parameters in broiler breeder hens.

iii. assess the influence of insemination frequency on fertility, hatching parameters and chicks quality from broiler breeder hens.

iv. determine the effect and suitability of dextrose and normal saline as semen diluents in broiler breeder hens.

v. assess the influence of dilution of semen on fertility, hatching indices and spermatozoa counts in the oviduct of broiler breeder hens.

1.3 Justification of the Study

Artificial insemination is unregulated in its administration in the poultry industry (Maeda, 2002). There is dearth of information on insemination schedule and the effect of oviductal sperm storage on fertility of eggs in broiler breeder hens in Nigeria. However, there is the need for certain bench mark in poultry production on the least viable semen dose and frequency of insemination required for optimum fertility. Therefore, saving on the cost of maintaining high number of male birds required for natural mating can increase the profit margin for farmers, if AI is adopted for broiler breeder chicken hens using least viable semen dose. The labour input required in the use of AI can be justified with the frequency of insemination of the hens and effective use of the cocks. A cock deposit much more semen during a single ejaculation during natural mating which lead to semen wastage and economic loss to the famers.

CHAPTER TWO

LITERATURE REVIEW

2.1 Breeds of Chicken

At the inception, varying number of breeds were cross-bred in the production of meat type birds in the broiler industry, with focus on colour sexing, growth rate and meat yield.

2.1.1 Barred Rock: In the early 1900's, the breed was utilised as a meat producer at the university of Cornell and Guelph. Characterized by a growth potential that compares as moderate to other breeds and dark pin feathers resulting from feather colour. The breed's exclusive use in commercial breeding programs for female lines, has been hampered by the bird's red/ brown plumage (Bri - Wyzard, 2020).

2.1.2 White Plymouth Rock: An eventual choice breed for female lines for a large proportion of breeding programs. Developed in the New England States in the1870's. Its white feather was a major merit for utilization. Initially a slow feathering birds, the fast feathering allele was introduced (Bri Wyzard, 2020).

2.1.3 New Hampshire: Early breeding programs utilised this breed for female lines because of its appreciable growth performance, egg laying ability and hatchability (The livestock conservancy, 2015).

2.1.4 White Cornish: From the 1920's - 30's the White Cornish, with its characteristics feather (white) heavy muscle broad breast and skin (yellow), presented immense potential for the establishment of white feather broiler. Thus the White Cornish assumed a pivotal role in male line under breeding programs (Skinner and Hady, 2018).

2.1.5 Light Sussex: The Light Sussex was originated in Europe in establishing a breed useful for either male or female lines (David, 2014).

2.2 Male Testes

Males of the avian species have testes positioned anteriorly to the kidneys at the dorsal aspect of the abdominal cavity, Size differentiation exist between the two testes in birds, with the right often smaller than the left (Briskie and Montgomerie, 2007), and both testes amounting for approximately 1% of the entire weight of the body, dependent on breed (Moller and Briskie, 1995). Lin and Jones, (1990) reported that testes comprises seminiferous tubules rooted in interstitial tissue which contains nerves, blood lymphatic vessels and Leydig cells, consisting of Sertoli cells and evolving germ cells. Attached to the testes are various of tubes (Vasa efferent), the epididymis, and empting in the vas deferens. The epididymis is made up of efferent ducts, rete testes, connecting ducts and epididymal ducts. Epididymal duct continues in convolution into the deferent duct, with a 3-fold diametric increase amid the cranial epididymal tube of the distals end (Maria and Barry, 2019). The tube deferent at distal end is uncoiled and enlarges at the dorsal juncture before terminating at the urodeum.

2.2.1 Spermatogenesis in Male Birds

Spermatogenesis is the process by which sperm cells are produced from progenitor diploid spermatogonia, which divide and go through mitosis to give two duplicate cells and successive primary spermatocyte before cleaving through first meiotic division to form haploid cells secondary spermatocytes. Which becomes spermatid via second meiotic division. The flux of testosterone, follicle stimulating hormones and their activities determine the process of spermatogenesis which is under the control of neurons (Sharpe, 1994). Spermiogenesis which describes the conversion of spermatid to sperm cells, goes through an 8- 10 step morphological process in the seminiferous epithelium (Tiba *et al*., 1993). Spermiogenesis results in cytoplasmic expiration, condensation of cell nucleus, preceded by the development of an acrosome and axoneme (Aire, 2018).

Spermiation occurs when seminiferous epithelium releases fully developed sperm cells into the lumen of the seminiferous tubules. The cells are suspended in Sertoli cell secretions and move through the tubules via fliud equilibrium hydrostatic pressure of the secretion and myoepithelial basket cell contractions (Archana and Bedwal, 1998). The movement of spermatozoa via excurrent ducts is measured to cover a couple of days and requires peristalsis when navigating the deferent duct (Troedsson *et al*.,

1998). Situated in the deferent duct is an extragonal reverse for sperm, with the highest absorption of spermatozoa (Allen *et al*., 1993). At spermiation spermatozoa possess no motility, but acquire this potential via movement through the excurrent ducts (Ashizawa and Sano, 1990).

2.2.2. Ultrastructure of Spermatozoa

Various descriptions have been ascribed to spermatozoa of avian species, depending on the equipment used. According to Abaigar *et al.* (1999) under the light microscope, avian spermatozoa are elongated flagella–like cells separated into a head, midpiece, and tail. On the other hand, Aire (2003) observing chicken spermatozoa via scanning electron microscope described them to be about 0.5 - 0.7µm in width and 90 µm in length. There are subtle changes in structure of rooster sperm while transmitting excurrent ducts. Bakst and Cecil (1997) carried out an in-depth examination of the structure of the head, neck and mid - piece of White Leghorn sperm via a transmission electron microscope. They noted that about 30 mitochondria occupied the midpiece.

Structural changes in the mitochondria of sperm in consonance with positive association found between mitochondrial status. The integrity of the mid-piece and potential for fresh semen to fertilize point to the sperm cell mitochondria as viable indicators of cell integrity and quality (Korn *et al.,* 2000).

2.2.3. Hormones in Male Chicken's Reproduction

Hormonal regulation start from the hypothalamus, responsible for gonatrophin releasing hormone. This hormone induces the adenohypophysis to release follicle stimulating hormone (FSH) and luteinizing hormone (LH). Male birds, growth differentiation and seminiferous tubular activity are activated by, luteinizing (LH) hormones while follicle stimulating hormone (FSH) attaches to receptor of Leydig cell surface and Sertoli cells (Vizcarra *et al*., 2010). In response to Luteinizing hormone, Leydig cells begin the production and discharge of testosterone and androstenedione into the circulatory system. Elevation of plasma levels of androgens in the blood stream bring about an inverse feedback mechanism which suppresses the discharge of gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) from the anterior pituitary gland and hypothalamus, Making both FSH and LH important for sperm formation (Braunstein, 1997). The functioning of the testicle is predicated by the activities of Follicle stimulating hormone and testosterone in combination

(Vizcarra *et al*., 2010). The effect of age on the neuroendocrine system ultimately impacts on male reproduction resulting in depression of the aromatase in producing estrogens, testosterone and vasopressin, all of which are essential for sexual activity in the male (Panzica *et al*., 1996) and indicating that older roosters might exhibit decline in libido.

Leydig cells are responsive to photostimulation and in the testes this induces production of testosterone and rostenedione, and their release into the bloodstream. Testosterone is essential for the stimulation of sperm production (Vizcarra *et al*., 2010) as well as the sustained activities of excurrent ducts, sexual behavior and characteristic of secondary natures and changes in the mode of GnRH discharge. Presence of testosterone can in-activate the gonadotropin releasing hormone GnRH mediated release of both luteinizing hormones and induce FSH secretion (Braunstein, 1997). Combs and wattles which represent sexual accessory tissues in male birds accumulate testosterone and convert it into 5-α- and 5-β- dihydro-testosterone (Martini, 1982). Plasma testosterone levels in meat – type breeding birds are highest at about 4 ng /mL at 30 week old and maintain an average of 2.5 ng /mL between the age 40 and 60 weeks (Hocking and Bernard, 2000).

2.2.4 Ambient temperature and its Effect on Sperm Storage

It is clear from the survival rates of sperm in the epididymis and the reproductive tract of female that sperm cells remain viable at body temperature for a significant period of time (Marire, 2011). Refrigerating semen at 5°C and freezing semen have greatly increased its *in vitro* storage life (Dumpala *et al.,* 2006). The need for unrefrigerated semen has therefore diminished, but in some areas refrigeration is not widely available. Hence storing spermatozoa in nutrient media at ambient temperature obviously makes it necessary to control bacteria. Adebisi and Ewuola (2019) used different diluents to extend life span of turkey semen and reported change in motility in 3 - 4hours while fertility was still sustained up till 4 weeks. When chicken semen (either undiluted or diluted) is stored close to the temperature of the females body 41°C (Tara, 1998), semen mobility is lowest. This contrasts storage at lower temperature of 25°C, 15°C, 5°C (Laffaldano *et al*., 2010). Storage of semen at 0-5°C for four hours reduces motility of raw fresh semen. Laffaldano *et al.* (2010) reported that motility and fertility

may be altered in cooled samples of semen stored in various materials such as bags, glass and plastic tubing.

2.3 Structure of the Oviduct

At early incubation female chicken embryos possess reproductive systems (ovary and oviduct) on both sides of the body cavity. By mid- incubation, regression of the right ovary and oviduct becomes evident, with the system remaining nonfunctional throughout the life of the hen. At maturity, the left ovary resides within proximity of kidney deep in the abdominal cavity (Husveth, 2011). About 20,000 eggs are ripen at maturity, of which around 2,000 are evident (Johnson, 2014). During maturation of ovum, sequence of eggs are supplied for everyday ovulation. The ovary of a matured chicken hen weighs about 36g, consist of 4 big ripening follicles, and a several 9-12 follicles size. The follicle mass, which compose of nearly 50% protein and 50% fat per unit of dry matter, is dropped in the latter 3 – 4 days preceding to the ovulated follicle. Regression occur when follicle did not ovulate which could lead to rupture or re-absorption back to the body cavity which can lead to peritonitis. Under-feeding or molting are common cause of regression of the follicle (Husveth, 2011).

The oviduct is in five major segment (infundibulum, magnum, isthmus, shell gland, and vagina), which when fully developed is 25 to 27 inches long. When follicles are released, they dropped into the infundibulum known as funnel form of the oviduct. The follicles that were unsuccessful to get to the oviduct are re-absorbed back into the body cavity which mostly transpires as multiple ovulation which is a more common incidence in broilers breeder.

In birds, fertilization takes place in the infundibulum, within a short fertilization window lasting between 15-30 minutes shortly after ovulation (Chiba and Nakamura, 2000). The infundibulum also contain albumen which is made inside the magnum the largest portion of the oviduct and measuring about 13inches in length. The magnum is responsible for deposition of the albumen protein. In the magnum over 3 hours period, the albumen forms around the yolk before passing to the mid- isthmus were deposition of the shell membranes take place. Both internal and external shell membranes are formed in the isthmus, where the developing egg resides for about 75minutes. The egg spends a major duration of period in the shell gland (uterus), Calcium carbonate is present as the major component of egg shell which is 8 to 10 percent and which

is mobilized from calcium deposits in the bones of the hen. About 47 percent of the calcium utilized in shell formation is derived from bone calcium, and at least another 20 percent from the duct of the hen. It takes over 15-18 hours before gradual deposition of shell material is completed. The pigments of the shell are lastly deposited in the last 2-3 hours before laying of egg. While it takes 4-5 minutes for egg formation process in the vagina, it plays an important role in egg laying process. The muscles that make up the vagina assist in pushing out the egg for oviposition. Close to the uterovaginal junction, were the uterus and vagina meet they are deep glands known as sperm storage tubule (SST) which can store sperm cells for lengths of period (Jacob, 2015).

2.3.1 Oviductal Sperm Storage

In poultry species, sperm storage tubules (SST) are found in the oviduct as a spermatozoa storage organ. Holm *et al.* (1996) first recognized the occurrence of anatomical structures 'sperm nests' related with elongated sperm storage in the infundibulum of the chicken. The utero-vaginal junction (UVJ) of the oviduct contains SSTs, where the sperm cells are deposited and stored (Bakst *et al.,* 1994). Histological studies of Bakst *et al*. (1994) indicated that infundibulum sperm storage sites are the secondary storage site. While primary sperm storage site is at the junction of the uterus and vagina. Sperm storage sites in the UVJ have been denoted as vaginal glands, sperm glands, UV sperm-host glands, sperm storage tubules and Uterovaginal sperm-storage glands (Ito *et al*., 2011). Spermatozoa are sustained in a movable state inside the tubules situated at the joint of the shell gland and vagina (Sasanami *et al*., 2013).

Sustained storage of spermatozoa by female birds following mating is well-known to take place in some species, with sperm storage durations ranging from about 8 weeks to 10 weeks (Adebisi and Ewuola, 2019a). Only approximately one percent of the deposited sperm travels via the selection process in the vagina to get to the uterovaginal junction UVJ (Khillare *et al.,* 2018). The length of sperm-storage in the SSTs is dependent on species. In hens, sperm could be stored for up to three weeks, where turkey hens can retain sperm for up to 10 weeks in the storage tubules (SST) and lay fertilized eggs (Brillard *et al*., 1998). In the uteroviginal junction (UVJ), spermatozoa enters the tubular invagination of the surface epithelium of the mucosa collectively called sperm storage tubules (SSTs) where as they are stored for a longer duration and also retaining their fertilizing capacity (Khillare *et al.,* 2018). This may be linked to number of SSTs present or stored in the uteroviginal junction (UVJ); turkey hens have

been reported to have about 20,000-30,000 SSTs, whereas chickens have been assessed to have just only 5,000-13,500 (Bakst *et al.,* 2010). In addition, after series of generations for selection of higher fertility, chicken hens possessed increased numbers of SSTs when compared to non-selected control hens, suggesting that the number of SSTs might be positively correlated with fertility (Brillard *et al.,* 1998).

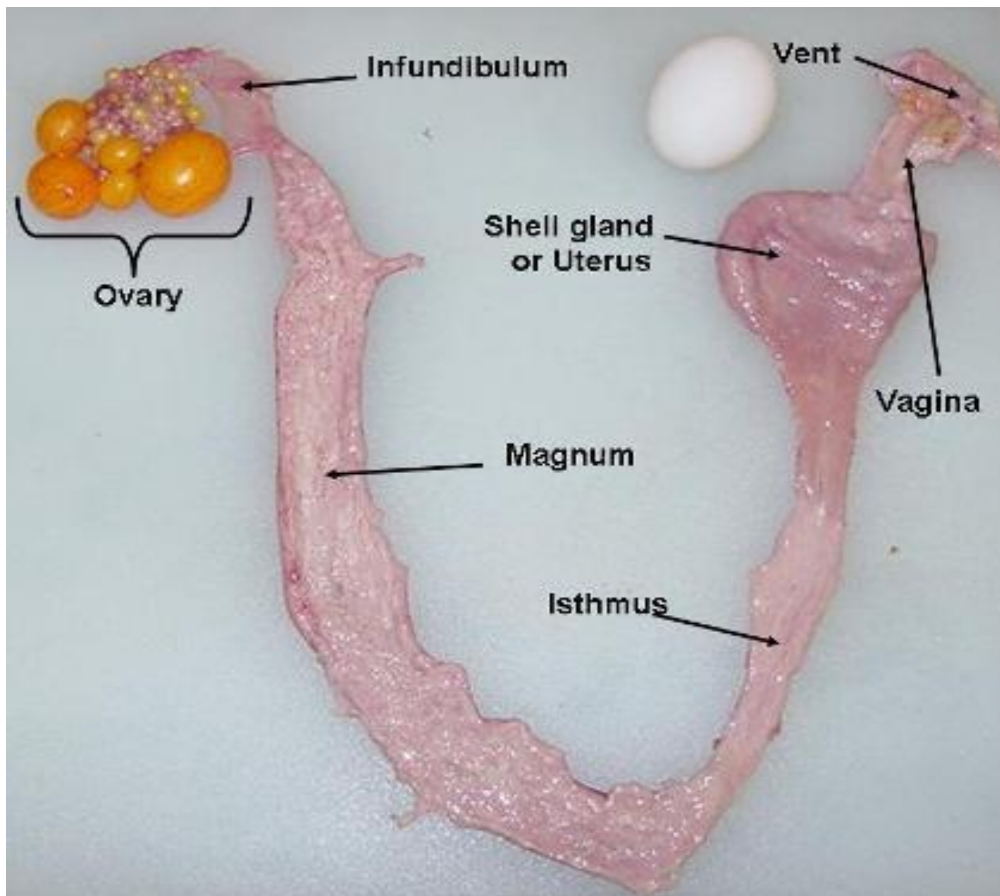

Figure 2.1: Avian Reproductive Tract

Source**:** Jacob (2015)

In contrast, similar numbers of SSTs indicated different fertility levels exhibited by different broiler strains under commercial conditions (Bakst *et al.,* 2010).

It is clearly shown that the SSTs create a discrete environment to sustain sperm viability via the inflow and outflow of compounds critical for sperm survival (Bakst and Dymond, 2013). The length of sperm storage is defined here as the length of time for which chickens hens could lay fertile eggs after the last insemination or post insemination. Though the duration of sperm storage can be different between bird species, the mode in which spermatozoa are stored and released out from the sperm storage tubules is unclear or poorly understood.

2.3.2 Ovulation

Breeder's birds do lay fertile eggs on a regular cycle with a few days of non-oviposition. Typically, production at highest or peak is between 85 and 87%, with intermittent flocks attaining 90% for 10- 14 days. The attainment of such production rates suggests an ovulation cycle approaching 24hours in birds Pituitary and follicular hormones control ovaries release of follicles; the process of which is mediated by lighting schedule. Under the influence of the light/ dark cycle, the brain mediates the release of luteinizing hormone, which influences hormone production during follicular maturation in ova (Cunningham *et al.*, 1984). As a result, progesterone output is increased. This secretion of LH by the brain takes place within a 6-8hours window every day. Failure to secrete LH during this period will put a gap in the clutch sequence and inadvertently a non-egg lay day (Ottinger and Bakst, 1995).

2.3.3. Length of Clutch and Ovulation Oviposition Patterns

Female meat type breeders like most avians lay eggs consecutively for some days, in what is called clutches. Clutch size in present breeder strains has become quite extensive, a result of selection for extended number of days with peak production. Clutches of eggs are parted by one or two days of non-ovulation, and also the interval of the clutch in relation to the non-egg days, connote the optimum possible rate of production (Jacob, 2015).

2.3.4. Ovulation as Influenced By Feeding

An undesirable characteristic occasionally exhibited by broiler breeder in response to feeding or energy excess is multiple ovulation. This occurs when the female release more than one follicle in a day. Multiple ovulation has been linked to obesity. The release of multiple follicles results in unsettable eggs due to the presence of double or even triple yolked eggs. On the other hand, multiply ovulation would yield inconsistent laying patterns as many as follicles are lost. For instance, releasing two follicle at a time will limit production and could, in extreme cases, lead to duplication or triplicate hierarchies in birds on almost *ad lib* feeding (Goerzen *et al.,* 1996). Besides the loss of settable eggs, erratic ovulation times would cause even normal eggs to appear at odd hours usually between 15:00 – 16:00 hours. This unusual egg lay time serve as an early warning indicator besides increase in body weight associated with multiple ovulation.

Shell defects could as well be as a result of multiply ovulation. Double eggs shell are as a result of 2 eggs being formed at the same time and bodily get in touch with one another in the upper side of the shell gland. By ways of inconsistent timing of eggs entering the uterus, shell formation (calcification) of shell might be half-finished or absent in a number of eggs. Inappropriately uneven Overweight hens have less spermatozoa in their sperm storage tubules, also lead to reduced activity in mating resulting in low fertilization (Hocking and Bernard, 2000). Multiple ovulation cases do take place in pullet / immature breeders that are obese at sexual maturity.

Matured birds usually weigh around 2.2 kg at 22 weeks that was the recommended body weights provided by some commercial breeding companies for their birds. In management situations birds that are slightly heavy +1.0kg have good laying pattern and particularly those peculiar to the tropics, weights of birds more than the recommended breeder's body weight could result to multiple ovulation and failure to attain peak of egg production (Hocking and Bernard, 2000). Similarly, during post peak of egg laying production, delay to carry out or practice a stepwise gradual reduction of feed commensurate with reduced needs is also attributed to multiple and irregular ovulation. In this later situation, erratic ovulation joined alongside leading to obesity can cause unexpected and drastic drop in post peak number of eggs. Heaviness, independent of body weight, does not seem to be an elemental factor in multiple ovulations, and because of this, it should be recommended that birds being too heavy

at point of lay (rather than being obese) is likely to be the leading cause of the problem. Taking correct, exact data records of matured weight of body and variance of such body weight is hence an essential diagnostic device under these conditions. The reports of Hocking (1996) documented a production ovulation early rate mean of nearly 1.9 follicles/day for birds fed free-choice during rearing. While birds limited to commercial feed standards had a rate of mean of 1.2 follicles/day whereas birds on in-between of restriction ovulated 1.4 follicles/day. The data recorded establish that pullets express certain multiple ovulations, which intensely increased if birds are overweight at maturity.

2.3.5 Fertilization

Van Tienhoven 1968, suggested that three specific conditions must be met in order to achieve successful fertilization of the avian egg. First, there needed to be a long fertilizable life span of the ova and/or sperm. We now know that the sperm cell does maintain a long fertilizable life span inside the hen's body (Brillard and Bakst 1990). Second, there must be accurate synchronization between copulation and ovulation. This is not entirely true due to the sperm storage capacity in the uterovaginal junction of the hen's oviduct. However, there is need to be some synchronization of sperm presence in the infundibulum of the oviduct at the time of ovulation. Third, copulation must occur regularly enough to ensure that somewhat fresh and viable sperm are available at the time of ovulation.

In addition to the conditions mentioned above, successful fertilization is the result of the completion of several other steps. Following successful insemination, sperm cells must be properly stored within the sperm storage tubules in the uterovaginal junction (Bakst *et al*., 2010). Sperm then must be transported up the oviduct to the infundibular region which is the site of fertilization. Once the sperm cells are located in the infundibulum, they must recognize the sperm binding sites on the perivitelline layer overlying the ovum. Following recognition and binding of the sperm cell to these sites, viable sperm are induced to acrosome react to digest a portion of the perivitelline layer through which the sperm cell may pass through. At this point the male gamete has gained access to the female pronuclei and syngamy of the male and female gametes could occur. Succeeding these, the avian egg has then been effectively fertilized and with the suitable incubational environments, development of the embryo can occur (Bakst, *et al*., 2010).

2.3.6. Supernumerary Sperm at the Germinal Disc

Ever since the turn of the century, scientists have shown a special interest in the germinal disc area of the ovum. The germinal disc of the hen’s ovum overlies the female pronucleus and pass through the perivitelline layer over lying the germinal disc region which is required for successful fertilization of the egg (Abaigar *et al.*, 1999). Looking at the lesser area occupied by the germinal disc compare to the entire ovum, it was believed that physiological polyspermy (or the presence of supernumerary sperm) was necessary to ensure syngamy. Although only one sperm is required to fertilize the egg, multiple sperm would increase the likelihood of a single sperm coming in contact with the ovum and penetrating its vestments at precisely the correct place and time. Historically, the presence of supernumerary sperm at the region of the germinal disc of the hen's ovum has been well documented. Multiple sperm pronuclei have been measured in the germinal disc region of the pigeon, the turkey, and chicken (Nakanishi *et al.*, 1990).

The occurrence of multiple sperm trapped in the outer perivitelline layer (Brillard and Antoine, 1990) and multiple sperm penetrating the perivitelline layer in the germinal disc region of the chicken ovum (Bramwell *et al.*, 1995) have also been well documented. However, the number of sperm reported to be present in the area of the germinal disc by these authors varies considerably, primarily due to the different measurement techniques used. Trapped spermatozoa could possess the capability to join and to go through an acrosome reaction, and may be in transit to a future site of attachment, binding, and/or penetration or death. The sperm cells that penetrate the perivitelline layer possess all the criteria necessary to reach the female pronuclei. Bakst *et al*. (1994) stated that sperm cells must enter directly into the germinal disc region of the ovum to ensure fertilization of the egg. Due to the large surface area of the hen's ovum, this led many to believe that there must be a preferential attraction to the germinal disc area in order to increase the likelihood of the occurrence of successful fertilization. This preferential attraction, then, was thought to be responsible for the large numbers of sperm reported to be present in the germinal disc region. In support of the concept of a preferential attraction of sperm to the germinal disc region of the ovum, several reports have been published (Bramwell and Howarth, 1992a). The study of Bramwell and Howarth (1992a) reported a significantly greater number of sperm

penetrating the perivitelline layer from the germinal disc region as compared to the perivitelline layer from adjacent areas of ova exposed to spermatozoa *in vitro*.

2.3. Embryonic Development after Oviposition

Development of embryo take place while the egg is still being made in the oviduct of the hen and that the broiler embryo consists of about 40,000 to 60,000 cells. (Fasenko, 2007) reported that there can be variants in embryo development which take place as a result of not being of the same parent strains and ages, this is the utmost common stage of embryo development at the period of lay. Gupta and Bakst (1993) reported that embryos of domestic turkey hens are at not fully formed stages of embryo development at the period of lay compare to domesticated chickens. For normal embryonic development, suitable incubation temperatures are provided (37.5°C). Fertile eggs must be provided with appropriate incubation environment, also the eggs must not be filthy up to the point when they are in the incubator. (The three possible stages of embryonic development; early, mid and late stages for the period of the 21 days of incubation). Part of the main causes that usually affect this stages is egg storage environment.

The following stages shows the developmental stages of the hen embryo from incubation to hatching (Morgan, 2015). The diagram is presented in Figure 2.2

Day 1: Presence of embryo
Day2: Growth of embryo
Day 3: Presence of blood vessels
Day4: Heart beating can be observed
Day 5: Eyes could be observed, head begins to develop
Day 6: Head continues to grow
Day 7: Head is very large – half embryo's length.
Day 8: Limb buds could be observed
Day 9: Limb buds continue to develop
Day 10: Pipping tooth can be seen
Day 11: Pipping tooth can be seen
Day 12: Beak can be seen
Day 13: Beak can be seen. Toes begin to form
Day 14: Beak, Feather follicles appear on the head. Embryo transforms into bird

Day 15: Scales and claws indicate rapid growth

Day 16: Develop bird and Claws can be seen

Day 17: Downs can be noticed on the head

Day 18-19: Development is complete and chicks enters final growth stage

Day 20: Turning is complete, yolk continues to be absorbed and pipping starts

Day 21: Hatching of chick

Day 22: Day old chick

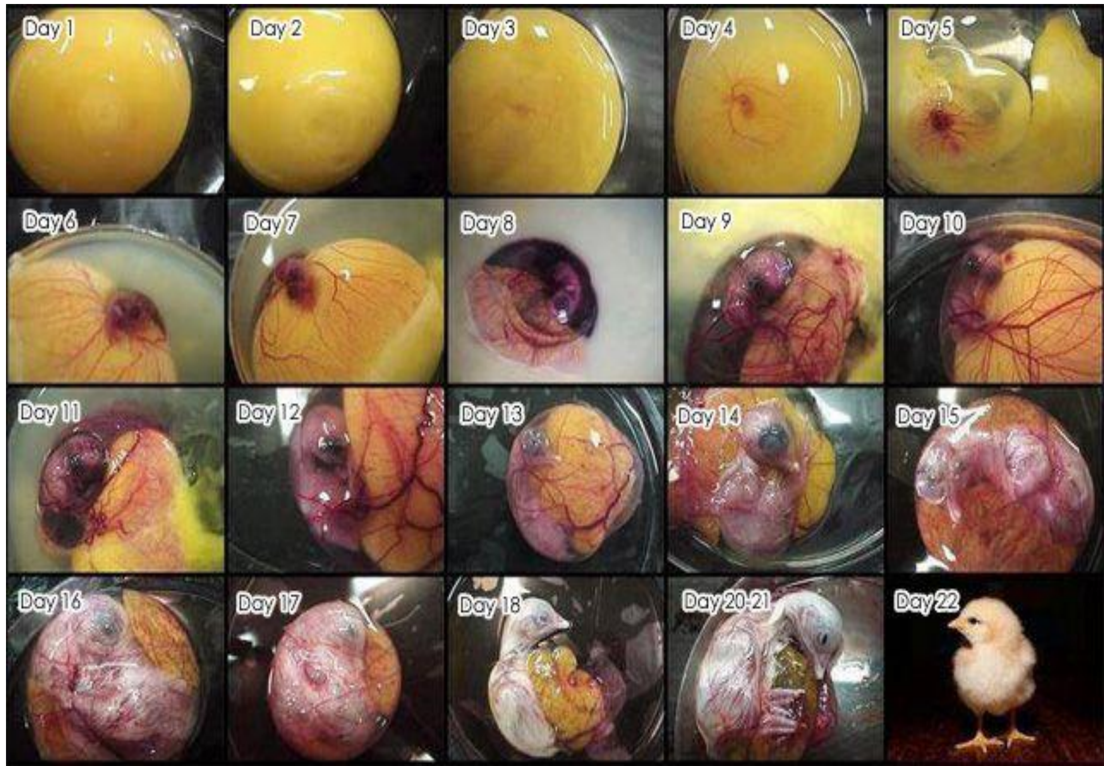

Figure 2.2: Embryonic Development in chickens

Source: Morgan (2015)

2.4 Fertility in Poultry Hen

Fertility in poultry production depends on viable eggs from female hen and viable sperm cells from the male chickens which fuses to form a fertile egg. To ensure 95 percent chance of fertilization, about 30 - sperm cells must get into the egg near the germinal disc. Although it is only one sperm cell that is required to fertilize an egg for fertilization to occur, the likelihood of an egg to be fertilized by one sperm cell reaching and penetrating it is actually low (Hemmings and Brikhead, 2015). In turkey hens, higher fertility is attained up till 3weeks post-insemination. It then declines slowly with some fertile eggs laid 10 weeks post insemination (Adebisi and Ewuola, 2019a). Only one insemination will be responsible for sperm cells to fertilize some numbers of eggs for some weeks. Nevertheless, for optimum fertility, insemination will be 3 inseminations in the first week, or 4 in 2 weeks. The second insemination should be carried out two days post insemination of the first and the third being not later than the end of the first week production period. A twice insemination in 7 days could be favourable to sustain optimal fertility (Tech. Advice sheet, 2006). The percentage of eggs laid can be affected by several factors namely: - Male to female ratio, Nutrition, Age of bird, Sperm quality, Breed differences and Temperature.

2.4.1. Decline in Broiler Breeder Hens' Fertility

Fertility of hens in terms of eggs production reduces after reaching a peak at about 35 weeks of age (Palmer and Bahr, 1992). Matured laying hens are considered to persistently have reduce fertility and hatchability after peak of production (Fasenko *et al*., 1992), with the most substantial drop in fertility at age 45 to 50 (Hocking and Bernard, 1997). Continual decrease in production of eggs and fertility in older hens can leads to major monetary losses for farmers in a breeder farm. Reduce capacity of sperm storage tubules for storing sperm cells could be liable for reduced fertility in older female hens (Hocking and Bernard, 1997). However, the study of Brillard (1993) using old and young hens showed that the number of spermatozoa in storage tubules in both treatment were not significantly different, although the number of sperm cells released in older hens double the amount produce by the younger hens, showing that more sperm cells are needed for fertilization to ensue in older hens. Maximum filling of the storage tubules takes about 1 to 2 days post-insemination (Bakst *et al*., 1994) and inadequate loading of the tubules could lead to decrease in fertility in mature

female hens (Walsh and Brake, 1997). Bramwell *et al*. (1996) reported the reduce fertility in older hens only viable for short period compare to sperm stored in younger hen. On the other hand, a research carried out on hens having short and elongated length of fertility showed that variances are not caused by different number of storage tubules. If adopting AI for broiler breeder in older hens, decline in female fertility as a result of age can be decreased by increasing the sperm number and by doubling insemination (Brillard and Bakst, 1990).

2.5 Fertility in Male Chickens

In breeder broiler male chickens, the reduction in fertility in broiler breeder male chickens can be as a result of aging (Creel *et al*., 1990), the use of artificial insemination could sustained fertility or introduction of younger male cocks into a pen of matured male called spiking (Sluis, 2014). In disparity, the report of Bramwell *et al*. (1996) shows that there is no variance in fertility between young chickens (39wk) and matured male (65wk) when both semen were evaluated and used for semen donor, signifying that sperm cells from matured cock maintain the functional and physiological ability to fertilise. Decrease in both sperm cell number and volume of semen are attributed to mature cocks (Rosenstrauch and Michael, 1994). Decline in production of spermatozoa can be due to alteration in the Sertoli cells that produce the consistence discharge of sperm cell (Rosenstrauch and Michael, 1994) otherwise reduction of action perceived in testosterone dependant structures are liable for reduction in production of sperm cells .

In matured male chickens, study indicated that ageing of sperm cells have low fertilizing ability (Creel *et al*., 1990). That could be attributed to a reduction in motility as a result of reduced polyunsaturated fatty acid in sperm cell (Cerolini *et al*., 1997). Uncontrollable body weight in a matured male chickens is one of the vital problems causing decline in fertility (Hocking, 1990) reason being that mating naturally can be actually difficult (Hocking and Bernard, 1997) for the heavy broiler breeder male chicken.

2.5.1 Comb Size

Size of comb has been a biological standard for level of plasma testosterone in matured male (Owens and Short, 1995) since growth of wattle and comb are male hormones

(androgens) dependent. Androgens are sex steroid hormones in the testes, linked with male sexual performance. McGary *et al*. (2002) reported that large comb size of broiler breeder male in a particular strain is an indication of high fertility potential. More so these investigator reported that specific type of breeder broiler male length of wattle correspond to sperm cell penetration of the perivitelline layer (McGary *et al*., 2003). Differing to the reports, observations indicated that colour and size of wattle and combs size characterized as external quality are not dependable features of semen volume (Parker and Ligon, 2007).

2.5.2 Aggressive Behaviour in Broiler Breeder Cocks

Males Broiler breeders are known to exhibit increased level of aggressiveness towards females (Mench, 1993). In a poultry house, the cocks will typically roam the pen area, while hens would be restricted to a raised section in the pen. Females that descend to the litter to feed are forcibly mounted after being chased. This increases injury to the hens and reduce fertility. Death of some hens also occur as a result of injuries. Aggressiveness as a syndrome is a problem in broiler breeder production due to welfare issues and reduced fertility in hens that are harassed, injured or even killed by the cocks. Initially, only one strain of broiler breeders seemed to be affected, but with time more strains were exhibiting the same behavoiurs (Millman *et al*., 2000). Aggression towards female by male could not be explained by increase in aggression, as game fowl male bred for fighting, with established aggression towards other male than is observed in broiler breeder, are hardly aggressive to female and even court female nicely (Millman and Duncan, 2000b). In production system, to maintain reproductive rigor, meat-type breeding males undergo severe restriction of feed, a factor known to elevate aggression in domestic fowl (Millman *et al*., 2000). When this was tested in broiler breeder males, no link was reported between feed restriction and increase in aggression, during rearing (Millman and Duncan, 2000b) and adult phase (Millman *et al*., 2000). In fact, fully fed males were more aggressive than restricted-fed males. It should also be stated that the problem definitely resides with the males; broiler breeder females respond normally to the courtship of laying-strain males (Millman and Duncan, 2000a). Contrarily, fully fed males exhibited higher aggression than feed restricted males. It is noteworthy that aggression is problematic in the male

breeder as breeder female show normal response to courtship display of egg - type males (Millman and Duncan, 2000c).

Meat-type breeder males have shown to be deficient in specific aspects of courtship behaviour. In comparison with egg-type male, in meat - type breeders waltzing frequency is similar while bitting and high – step advancing have lower frequency (Millman *et al.*, 2000). Thus the resultant effect is having females inappropriately retreating when the males advance (Millman and Duncan, 2000c), which could probably infuriate the males leading to greater frustration and aggression. While the source of aggression in meat-type male has not been elucidated, it is certain that it is genetically based. Also unclear is if the deficiency in courtship and the heightened aggression are mutually exclusive associated problems. There is a possibility that these traits are tied genetically to a production trait, such as broad-breast, a priority selection trait for breeding. Conversely, it may be the product of a misguided trials by geneticists to improve the relatively poor fertility, in broiler breeders, especially as the breeding year winds up. As has already been stated, there is a misconception in the industry that this fertility is as a result of decrease in libido. If the breeders firms have been selecting and crossing males that approach females very fast in the mistaken belief that this is a sign of sexiness, they may have been selecting males that are, in fact, very aggressive. The fact that male broiler breeders are deficient in bitting, a courtship behaviour pattern that involves moving away from the females, is evidence that points in the same direction.

2.5.3 Body Conformation and Fertility

The outcome of the increased selection for fast growth and larger breast between the 1970s and 80s was severe fertility problems in meat-type broiler breeders after 50weeks (Kirk *et al.*, 1980). There are several reasons that could be adduced for this such as body conformation of male which may hinder copulation. Duncan *et al.* (1990) investigated the decrease in fertility in broiler breeders at the later part of the laying year by monitoring sexual behaviour and fertility at 28, 38 and 58 weeks of age. The males in this study were also fed different energy levels in a bid to attain various target body weights. The occurrence of sexual behaviours, including courtship and copulations, exhibited a significant age – related decrease, which suggested libido decline. However, rather unexpectedly, the heavier males showed less of a decrease in sexual behaviour than lighter males. Moreover, the reduction in sexual behaviour

between 28 and 38 weeks of age had little effect on fertility, which was comparable with commercial fertility rates. However, there was a big drop in fertility at 58 weeks, particularly in groups with very heavy or very light males. The birds were inspected regularly and musculo-skeletal disease was ruled out as a cause. The very light-weight cocks also showed a bigger decline in courtship at this time and it was concluded that their energy intake was too low to maintain full sexual behaviour. The heaviest birds also showed a decline in courtship, but, rather unexpectedly, this was less of a decline than in the other groups. Being ‘overweight’ by industry standards did not result in a drastic reduction of libido. In addition to showing more courtship than the other groups, they were also seen to mount and tread the females more. The reduction in their fertility was therefore due to their bulk or their conformation precluding cloaca contact. Hocking and Bernard (1997) observed that body structure depended on breed of chicken which influenced fertility under natural mating, and the authors corroborate this to the Cornish breed’s unique body conformation and not to body weight.

When artificial insemination was introduced there was only a slight relationship between fertility and physical characteristic implying that reduced fertility was not a result of drop in semen quality but, rather inability to mate successfully. They were of the opinion that broad breast in combination with wide hips might make mounting difficult or increase incidence of weakness in the leg ultimately hindering successful mating. More probably, a broad breast in combination with wide hips will negatively affect pelvis girdle flexibility and prevent contact with the cloaca when the cock thrust downwards. It is well established, that later in the breeding year fertility in broiler breeding drops coinciding with broader breast and wider hip. Hocking and Bernard (1997) further corroborated the effect of broad breast and reduced pelvic flexibility. By manipulating body weight in two broiler breeder male strains they observed that a bigger breast muscle weight was linked with a lower fertility which was linked with an increased number of incomplete mating.

In a study, Hocking and Bernard (2000) investigated the effects of fertility and hatchability. They observed that the younger cocks mated more frequently, without any effect on fertility. Also, age did not affect the frequencies of courtship behavior in males. A common practice used to improve fertility of broiler breeder’s chickens is ‘spiking’ (Leeson and Summers, 2000). However, the introduction of sex-based

feeding has reduced this practice. Improvement in fertility could be linked to the triggering of the Coolidge effect as a result of placing new cocks with unfamiliar hens.

2.6 Natural Mating

In both sexes of young meat – type breeding chicken, natural mating is high and decline as the bird's age (Duncan *et al*., 1990). Successful copulation has been observed to be reduced in overweight breeder, indicating that body weight influence fertility decline (Hocking and Bernard, 1997). Lower fertility is related to high body weight gain in male birds leading to incidence of destructive cartilage loss, dyschondroplasia and ruptured ligament making mating naturally with remain challenging for the heavy male birds. Strains of birds can be associated to natural mating difficulties. Observation indicated that ability of overweight male chickens to fertilize hens naturally remained lower than light weight males (Hocking and Bernard, 1997).

2.6.1 Mating Ratio of Breeder Chickens

In broiler breeder flock mating ratio recommended for optimal fertility is 10 males to 100 females (1:10) of about 20 to 30 weeks (Ross Breeder Limited, 2018). However, this estimate was made for traditional breeder flocks of several thousand birds from their results, it was predicted that fertility could be maintained at a high level with a ratio of 1:10 or 1:11 cock: hens, but this prediction would probably only work in groups with one male present. The problem then is that if something untoward happens to that male (he becomes lame or injured or ill) there would be a catastrophic decline in fertility in the hens. The industry has therefore spread the risk and opted for a system with many hens and cocks and a ratio, reached by trial and error, of around 1:10. Of course, the effective ratio under commercial conditions is probably nearer to 1:20 or perhaps even higher. It is likely that many cocks in breeder flocks will not be inseminating hens. There will be males present who are prevented from mating by dominant flock-mates. There will be males who are psychological castrates. There will be males who are rejected by the hens. There will be males who are infertile for a variety of reasons. It has been well established that meat strains of domesticated fowl are less fertile than egg-laying strains (Hubbard Farm, 1996).

2.7. Artificial insemination (AI)

Artificial Insemination (AI) is the process whereby extracted semen from the cock is artificially inserted into the reproductive tract of the female artificially to produce conception. The earliest recorded of Artificial Insemination (AI) was by Spallanzani in 1780 an Italian physiologist. In the 19th century success in, Artificial insemination had been reported by Bakst and Dymond (2013). Artificial Insemination has been used in farm animals around the world. In poultry AI is a mechanism for inducing fertility by the introduction of sperm cells into the cloaca for the purpose of breeding. In United State of America turkeys produced annually for consumption are products of artificial insemination (Tara, 1998).

Donoghue and Walker (1999) indicated that artificial insemination (AI) of turkey is much more economical and effective than mating naturally looking at the total number of hens that are needed to be inseminated. An additional important benefit of artificial insemination in turkey is that smaller number of toms are required to maintain hens producing fertile eggs.

2.7.1 Method of Semen Collection

There are 3 semen collection approaches that are being used effectively in poultry. The first, is a co-operative method, which was initiated by falconers with sexually imprinted birds-of-prey and vagina. With this approach, birds willingly copulate on a specially made object in reaction to a behavioral stimulus. There is no handling of animal, no strain and risks of disturbance are minimised. The merit of this technique is that the sperm ejaculated generally is not severely polluted by urine. Nevertheless, seminal volume differs significantly amongst species, and some birds carry out copulatory actions, but failed to ejaculate or produce little or not one sperm cell. Innovative results have been manufactured by using an artificial vagina in the ram (Wulster-Radcliffe *et al.*, 2001).

The second process is electro ejaculation that has been used for ducks and geese (Frediani *et al.*, 2019), different types of parrot and pigeons. Though safe, when carried out appropriately, anesthesia is necessary, and ejaculates regularly are polluted with urine.

The third process is abdominal massage Adebisi and Ewuola (2019a) which described this technique of poultry semen collection. The cock is restrained or calmed, followed by massaging of the dorsal abdominal part. After which the handler smoothly squeezes

the cloaca, for semen to come via the external papilla of the ductus deferens. Collection of semen using abdominal massage for small birds is relatively easy (Birkhead *et al.,* 1995). Slight modification was made to this method in the area of how best to restrain turkey for semen collection Adebisi and Ewuola (2019a).

2.7.2 Measurements of Semen Quality

2.7.2.1 Sperm concentration: Chicken semen is thick and sticky, with broiler breeders having a high sperm concentration of about 2 billion to 4 billion sperm per ejaculate (Donoghue and Wishart, 2000). Sperm concentration is an essential indices of semen quality and give information on the extent of dilution needed to obtain required sperm numbers per insemination dosage. Haemocytometer can be used to measure concentration (Ewuola and Egbunike, 2010). Another system that is also used in measuring sperm concentration in poultry is the microprocessor controlled semen analyzer (Densimeter, Model 534-B-Model,Animal Reproduction systems) initially designed for assessing stallion semen but modified to accommodate poultry semen (Donoghue *et al*., 1996). This method provides accurate and precise data on sperm concentration beneficial to artificial insemination. Etches (1998) noted sperm cells concentration in semen of domestic birds which is higher than that of cattle, pigs sheep and goats. Broiler breeder semen volume 0.24-0.80ml while concentration is between 2 to 4 billion cells/ml (Donoghue and Wishart, 2000).

2.7.2.2 Sperm Motility: Sperm motility the movement of group of sperm cells in a certain direction. This considers that only sperm cells progressing in a straight line are counted, and excludes cells moving in a circular or backward direction. Sperm motility enables the measurement of sperm cells ability to swim in a solute at body temperature, in order to replicate the ambience of the female reproductive tract (Tara, 1998). Donogue *et al.* (1995) reported that it is likely that sperm motility influences the number of sperm cells in storage-tubules of hen and rate of the sperm cells that fertilize the egg. Motility can be estimated by dropping a raw semen on a sterile slide and a drop of warm sodium citrate of the same temperature with the semen viewed at 400x magnifications (Bakst and Cecil, 1997). Froman *et al*. (1999) observed that sperm motility can be a determinant of fertility in broiler breeders.

2.7.2.3 Semen Volume: In semen evaluation, the importance of semen volume cannot be over emphasized. This is necessary to calculate the appropriate rate of dilution to obtain the required number of cells per insemination. Variations in ejaculate volume of birds usually exist between species, breeds and individuals within a flock. Other factors include, age, frequency of ejaculation, season of the year and extent of massage or handling (Etches, 1998). The volume of semen of cocks ranges from 0.24 to 0.80ml, thus indicating that domestic birds produce small amount of semen compared with other farm animals. The volume of turkey semen ranges between 0.2 to 0.5ml. Small semen volume is not unsafe but then if accompanied by a small concentration of sperm cell, then the number of sperm available is limited. Turkeys ejaculate less semen volume compare to chickens but the concentration of spermatozoa is much more. Broiler breeder semen ranges volume from 0.24-0.80ml (Donoghue and Wishart, 2000).

2.7.2.4 Semen pH: Fresh semen can be alkaline with an average pH ranging from 7.0 to 7.6 (Froman *et al*., 1995). During storage, semen pH depends on the various proportions of several secretions in the semen especially the seminal plasma. For instance, semen containing bacteria and many dead spermatozoa as contaminants may evolve ammonia which decreases the pH of the samples (Donoghue and Wishart, 2000). Semen pH decrease during long term storage of sperm cell, thus resulting to a decrease in spermatozoa motility. This could be as a result of an increase metabolic rate leading to production of lactic acid anaerobically. Semen pH has be determined using pH meter and bromothymol blue paper.

2.7.2.5 Sperm Viability: Eosin and nigrosin and tryphan blue reagent are nuclei acid stains that are in semen sample to measure the percentage of life to dead ratios. In assessing semen quality, dead sperm cells and moribund cells in a sample can be separated by using an important stain in addition with nuclei acid stain (Bayyari *et al*., 1990). Spermatozoa viability can be measured by using a device known as flurometer (Januskanska *et al*., 2001). In determining flurometer, an insertion of ethidium bromide is used to infiltrate impaired spermatozoa and the viable sample is equivalent to the ratio of the initial fluorescence to calculate after all cells are destroyed with a steroidal saponin digitonin.

2.7.2.6 Spermatozoa Morphology: Avian spermatozoa according to Etches (1998) is about 0.5μm at its widest point and approximately 100μm length with a volume of about 10μm^3.The spermatozoa of avian species are lengthier compare to the mammals, but the head is thinner. Chicken and turkey spermatozoa are filiform in shape (Bayyari *et al*., 1990) under light and electron microscopy they are indistinguishable. They have simple acrosome with the mid-piece being cylindrical, of distal centriole surrounded by a cover of mitochondria. The chemical and physical properties of avian semen differ from those of mammals possibly because of the nonexistence of seminal vesicles and prostate glands in avain species.

2.7.2.7 Sperm Abnormality: Several deviations from the normal sperm morphology are regarded as abnormalities (Siudzinska and Lukaszewicz, 2008). These are classified into three categories based on their source and nature of incidence. List of the categories are provided:

2.7.2.8 Primary Abnormalities: These are due to defects in the seminiferous tubules and caput epididymis. Examples include structural deviation of the head, the middle piece and immature sperm cells.

2.7.2.9 Secondary Abnormalities: These occur due to degenerative changes in the sperm probably due to prolonged stay in the caudal epididymis. Degeneration of sheath lining the head and mid-piece, broken necks, detached heads, and burst heads are examples of secondary sperm abnormalities.

2.7.2.10 Tertiary Form: occur due to poor or improper laboratory handling of semen. Examples of tertiary forms of abnormalities include broken tail, coiled tails and broken necks formed as a result of faulty dilution, cold shock and crushing spermatozoa.

2.7.3 Pooling of Semen

The practice of pooling semen from several males in the field is an acceptable procedure since the semen quality characteristics are not seriously affected (Adebisi and Ewuola, 2019a). Pooling of semen helps to remove the effect of individual unevenness of gamete donor (Taner and Ergum, 2010). In artificial insemination pooling of rooster semen was encouraged because roosters are assessed independently for potential of reproductive performance at the period of semen collection further than evaluation of semen volume and colour (Holsberger *et al.,* 1998). When collected

semen from 7 to 10 toms were pooled together, only about 1 or 2 males produced a larger number of the offspring (Tara, 1998).

2.7.4 Semen Storage

Semen storage refers to the technique of storing or preserving semen for use during Artificial insemination. According to Carmen *et al*. (2004) Semen could be frozen and thawed. Graham *et al.* (1982) used some extenders for turkey semen with a frozen-thawed recovery of greater than or equal to 50% motile sperm cell and a vigorous swirl but detrimental effects occurred after some hours. In a study on semen preservation, Robinson (1996) observed sperm survival and hatchability when broiler breeder semen was put in short storage before artificial insemination. Liquid storage of more than 6 hours or freezing of semen has not well-preserved the viability of turkey semen at the level required for profitable use (Thurston, 1995). Gadea (2003) also reported that conserving semen at temperatures lower than 15°C could decrease sperm metabolic activity and guard against the harmful effects of microbial contamination. Several factors must be taken into account when preserving spermatozoa; these include temperature, energy source, osmotic pressure, electrolyte balance, pH and buffering capacity, microbial control and appropriate dilution rate (Froman *et al*., 1995).

2.7.5 Sperm-Binding Assay

A method was developed by (Bramwell *et al*., 1995) to enumerate the number of sperm cell that enters the perivitelline layer of an egg so as to know the capacity of the fertilizing ability of each cock. For the evaluation, the perivitelline layer of the germinal disc is removed, wash in sodium chloride in water riveted by means of 20% formalin, is stained using Schiff's - reagent. Saline solution was used to rinse the excess Schiff's reagent and formalin from the slide. A sterile cover slip was used to cover the slide and the glass slide is blown dry. A white colouration appears on a dark purple surface of periviteline layer as a result of holes initiated by the spermatozoa. This can be enumerated and viewed under light microscope using a magnification of x100. The age of birds can negatively affect spermatozoa penetration of the perivitelline membrane (Bramwell *et al*., 1996). Though Barbato *et al*. (1998) brought about sperm-binding experiment. The perivitelline layer of freshly unfertilized egg is isolated and solubilized in heat. A spermatozoa suspension is dropped into a flat-bottomed microtiter plate containing the heat solubilized perivitelline membrane and

incubated at 35°C for 180 minutes. The layer decanted, washed and stained using 4’, 6’-diamidino-2-phenylindol to count bound sperm cell. Spermatozoa binding is reported per square millimeter under light microscope. Spermatozoa egg binding assay showed high relationship (r = 0.83) towards fertility (Barbato *et al.*, 1998).

2.7.6 Spermatozoa Quality Analyzer®

The spermatozoa quality analyzer® (SQA) according to Bartoov *et al*. (1991) was planned for assessing spermatozoa quality in human. In experimentation, 3 - 5 fold of semen diluted is drawn in capillary tube, and retained inside SQA for about 40 seconds, which give sperm motility index which give expected viability, sperm concentration and sperm motility (McDaniel *et al*., 1998). In the same laboratory Parker *et al*. (2002) reported that choosing of male depend on sperm motility index as young age which could increase fertility. Report of Froman *et al*. (2003) established that Avi Mate TM SQA was at variance with sperm mobility physical trait. That is sperm quality assay did not predict sperm mobility phenotype.

2.7.7 Computer Assisted Sperm Analysis (CASA)

Computer technology has permitted objective spermatozoa counts and motility determination in human beings with positive relationship for *in vitro* and *in vivo* fertilization experiment (Barratt *et al*., 1993). The device Hobson Sperm Tracker® analyzed the motile sperm velocity and sperm concentration parameter in swift animal (Abaigar *et al*., 1999) and in chickens and turkey hens (Froman and Feltmann, 2002). The speed at which spermatozoa travelled was found to be different between high and low sperms motility in chicken (Froman *et al.*, 1999) and turkey (Donoghue *et al*., 1998).

2.8 Sperm Egg Interaction

Two unrelated methods of assessing sperm quality have been developed based on sperm interaction with the egg investments, the outer and inner perivitelline layers. One method involves assessing the capability of sperm to adhere to solubilized (outer and inner) perivitelline proteins in a multi-well plate and assessing the number of sperm bound by reading the fluorescence after staining with a DNA-specific fluoro-chrome (Barbato *et al.*, 1998). The second procedure is based on the finding that sperm

can produce, *in vitro*, points of hydrolysis in the inner perivitelline layer of ova removed from the body cavity or infundibulum (Bramwell and Howarth, 1992).This was later characterized as an assay measuring sperm interaction with pieces of inner perivitelline layer separated from laid eggs, and quantified as the number of points of hydrolysis per unit area (Robertson *et al.*, 1998). Whatever the application or parameter, sperm quality tests should ideally be able to predict fertilizing ability, or be shown to be correlated with fertilizing ability, or at least should be able to rank birds according to the measured parameters on the assumption that these are likely to reflect fertilizing ability.

In both mammals and birds, tests of semen quality have often been considered to be poor predictors of fertilizing ability. However, the research documented below clearly demonstrates that, in poultry, sperm quality tests are highly significantly correlated with fertilizing ability. The general principle of sperm quality tests is that they should, as much as possible, reflect the various functions that spermatozoa must display during their interaction with the female reproductive tract and the ovum. In birds, this argument would also seem to be valid and these functions would include (Bakst *et al.*, 1994): (i) transport through the vagina and entry into the (SSTs), requiring motility and perhaps appropriate surface proteins and glycoproteins; (ii) maintenance in the SSTs, which may depend on internal substrates or a response to regulatory signals; (iii) exit from the tubules, which may require increased or decreased motility (Froman, 2003); (iv) transport from the uterovaginal junction to the infundibulum, which may be passive, dependent on uterine contractions; (v) inner perivitelline-layer binding, acrosome reaction and hypermotility for penetration of the inner perivitelline layer; (vi) binding to proteins for the oolemma receptors for engulfment; and (vii) the ability to form a male pronucleus with an intact genome.

Additionally, response of hens to inseminated spermatozoa varies considerably when considering ratio of fertile eggs produced by hens when specific sampled aliquot of semen are utilized (Wishart, 1995). Relationships have been found between individuals chicken males fertilizing ability sperm motility and ATP (Packer and McDaniel, 2006), tetrazolium dye reduction (Hazary and Wishart, 2000), and spermatozoa mobility (Froman *et al.*, 1999). Therefore, in chickens, single-parameter sperm quality tests can be shown to correlate with sperm fertilizing ability, so either

the avian system is less complex than that of mammals or the avian system for measuring ‘fertility’ is more quantitative.

2.8.1 Sperm Quality as a Trait and Heritability

The various sperm quality parameters: sperm mobility (Froman and McLean, 1996), sperm ATP, motility and perivitelline layer hydrolysis (Robertson *et al.*, 1998), are traits that are repeatable within ejaculations from individual birds, and sperm mobility differences are maintained throughout 20 weeks of the breeding season (Froman, 2006). Furthermore sperm mobility among males is normally distributed, with an approximately tenfold range (Froman and McLean, 1996). Extrapolating to all assays, sperm quality appears to be an individual male trait, which raises the question regarding its heritability. Heritable reduction in sperm quality do exist in Delaware strain. For example, the fertile Delaware strain, in which the lesion is linked with aberrant morphology of male excurrent duct (Kirby *et al.*, 1990). The hypothesis that the primary lesion for poor sperm quality might derive from sperm oxidative metabolism (Froman and Feltmann, 1998), combined with the knowledge that mitochondrial genes, which code for components of the electron transport chain have been implicated in mammalian infertility (Gemmell *et al*., 2004). Froman *et al.* (2002) performed experiments that suggested that the heritability of sperm mobility might have a significant maternal influence. Furthermore, a single nucleotide polymorphism (SNP) identified by restriction fragment length polymorphism (RFLP) analysis was later shown to be associated with low but not high sperm-mobility males (Froman and Kirby, 2005).

2.9 Semen Extender

Semen diluent or extender is a chemical substance added to semen to conserve it fertilizing capability. Semen extenders are different in chemical component, but all contains constituents, that serve the same purpose. It contains a cryopreserving agent, nutrient, buffer and antibiotics. A typical example of nutrient used is a sugar, such as sucrose or glucose that serve as energy source for the spermamatozoa. Buffers are supplemented to balance the potential of hydrogen pH and osmolality of the solution, and the sugar also serve this purpose. Glycerol is usually use as cryprotectant, though others likes dimethylsulphoxide (DMSO) have also been used with varying success. Cryprotectant, assist in stabilization of the cell during freezing and thawing process.

Egg yolk, which is a commonly used constituent in a wide range variety of frozen semen extenders, serve numerous purposes. It has nutrient value for sperm during the freezing and the thawing process; It acts as a buffer to some extent and likewise most essentially coats the sperm cell, and this prevents alterations to losses of some of lipo-proteins present in the cell membrane that occur during the freezing and thawing processes. Different antibiotics are mixed with semen and added to control bacterial content that could be present in the raw semen. It should be well-known that the antibiotic will only protect against bacterial and not viruses, when is being frozen in liquid nitrogen which is an ideal storage medium. Lukaszewicz (2002) has recommended these criteria for a satisfactory extender. An extender must;

1. Provide nutrients for the sperm during storage
2. Protect against cold shock,
3. Contains antibiotics to inhibit bacterial growth,
4. Buffer the extended semen against changes in pH,
5. Maintain proper osmotic pressure
6. Increase the volume without decreasing viability thereby making semen easier to work with.
7. Protect the spermatozoa all through the freezing and thawing process

In addition to providing a satisfactory environment, an extender must be able to sustain sperm viability during *in vitro* preservation to be of any usefulness during insemination. Also semen extenders are categorized into short (1-2 days), medium (3-4) or long term extender (5-7 days) from the day of collection (Lukaszewicz 2002). The adequate extension of semen is a very important stage in attaining success in fertility with AI. While the technique is not hard, precise dilution rates are important for sperm survivability in storage. In adequate extension could result to production of greater amount of toxin products from sperm cell metabolic by- products. By the time diluent, or extender, are used to increase the volume of the ejaculate to meet the necessary doses. While doing this, preservation of the structural and functional characteristics of the sperm cells is important to maintain good fertility rate. Therefore, to conserve spermatozoa for a lengthy period of time, their metabolic action needs to be reduced by diluting it in a suitable medium and by lowering the temperature.

2.9.1 Constituent of Diluents

A semen extender is expected to provide the nutrient required for the metabolic sustainance of the sperm-cell (glucose), afford defense against cold temperature shock (BSA), to control the potential of hydrogen pH (bicarbonate, Tris, Hepes) and osmotic pressure (NaCl, KCL) of the medium and prevent microbial development (antibiotics). The spermatozoon could provide the energy required to sustain its cell metabolic rate and cause the mobility of the flagellum, mostly via glycolytic path-ways. These processes transpire in the mitochondria situated in the middle portion of spermatozoon. The energy source usually used in semen diluent is glucose, even though some sugar have been verified which includes galactose, fructose and ribose or trehalose but are known to have produced poor outcomes.

2.9.2 Regulating pH of Diluent

During pH reduction, sperm motility and metabolism are both reduced. Its glycolytic, metabolism (glucose is the key carbohydrate) resulted to a reduction in intra-cellular pH and as a result, cell metabolism is suppressed. Lactic acid is the key metabolite of this process and has been used as factor of indicating semen quality Riqau *et al.,* (1996).

The adding of buffer as an agent assists to regulate the pH of the medium. The common buffer used are bicarbonate and sodium citrate, which display a limited buffering ability. Other more complex buffer (TES, Hepes, MOPS, Tris) could regulate the pH over a varied range and are not temperature-dependent (MOPS and Hepes).

The pH of diluent usually ranges between 6.8 to.7.2, but it must be well-known that in the media, the pH does not turn out to be stable from the start of dilution in water until 60-90 min. Also, various extenders display a diverse pattern of pH changes with time (Newth and Levis, 1999). Hence, suitable procedures needed to be engaged when preparing the diluent to circumvent harmful effects on preservation.

2.10 Semen Dilution Ratio

Obvious advantages of semen extension have generated interest in finding dilution rates for semen of avian species. Donogue and Wishart (2000) advanced that the ability to obtain fertility results similar to natural mating was dependent on the rate of dilution. Adebisi and Ewuola (2019) stated that a weekly insemination in turkey at a dilution ratio of 1:3 could result in high fertility. Dilution levels of 1:5, 1:10 and 1:20 exhibited

marked reduction in fertility compared with 1:2 and 1:3 and not even an increase in semen dosage form 0.1 to 0.2ml improved fertility in chickens at the 1:10 and 1:20 level because of the dilution effect. Omprakash *et al.* (1992) observed an increase in abnormal sperm percentage with semen diluted at 1: 3 ratio.

2.10.1 Timing of Insemination

Insemination timing in birds is very crucial in attaining high fertility levels. As a general rule, insemination is best when the uterus is expected to be free of hard shelled egg or after oviposition has occurred, for at least 3hours (Obidi *et al.*, 2008). Good fertility can occur during insemination even when an egg in the uterus is undergoing plumping. The occurrence of a hard-shell egg in shell gland may impede insertion of the semen and also lead to washing out of spermatozoa if ovipostion occurs after insemination prior to sperm gaining entry to the storage glands. Chickens are inseminated at weekly intervals, whereas the turkey requires insemination at 2 to 3 week intervals (Adebisi and Ewuola, 2019). Marire (2011) pointed out that insemination of chickens twice weekly could lead to maximal fertility. But for good results in turkey insemination of hens at 1, 2 or 3wks interval is ideal. Hens are inseminated late in the afternoon between 2 to 3p.m to avoid the occurrence of a hard shelled egg in the shell gland also known as uterus.

2.10.2 Insemination Dose of Semen

Standard doses in the industry contain about 100 and 200 million sperm cells when inseminating chickens and turkeys respectively (Etches, 1996). For older hens bi-weekly inseminations, or once weekly with 250 million sperm will maintain fertility sufficiently (Brillard and McDaniel, 1986). Spermatozoa quality is a greater factor limiting fertility with regards to AI, than inseminated number of spermatozoa as well as oviduct selection (Froman *et al.*, 1999). Thus, in order to determine difference in sperm quality between males, spermatozoa inseminated in minimal or low doses is sufficient. The semen doses that can effect fertility vary according to species. There is an inverse relationship between the concentration of sperm cell and the required dosage. Available information revealed that different dosages had been used to effect fertility in poultry and other animals, Lukaszewics and Kruszynski (2003) reported

that the use of 0.2ml dosage obtained fertility result of 66.1% in Turkey, 82% fertility result was reported in duck using 0.3ml (Bunaciu *et al.,* 1990).

2.11 Duration of Fertility in Broiler Breeder Hen

The potential for fertilization after a single insemination can be assessed via fertility duration (Kirby *et al*., 1998). During a period of 2 - 21 days after insemination, fertility is expected to decline in a sigmoidal pattern (Kirby *et al*., 1998); Rapid age – related decline indicated lowered quality of semen in male birds (Bramwell *et al*., 1996). Observation of flock at 39 and 59 weeks of age, however showed no difference in fertility duration, indicating that age of birds had no influence on fertility duration (Fairchild *et al*., 2002), Fertility duration can be 22 days in the domestic hens (Bramwell, 2002). Studies have highlighted that hen weight was negatively correlated with duration of fertility (Goerzen *et al*., 1996). In addition, strain of birds has been observed to differ in duration of fertility in breeding lines of commercial stock (Tabatabaei *et al*., 2009). Contrastingly Kirby *et al*., (1998) reported no broiler breeding line effect on fertility duration.

2.11.1 Assessing Fertility

At the hatchers, fertility can be determined either before or after hatch of eggs. Failed fertility can be seen in candling or hatch failures like setting of eggs upside down, cracks in eggs and dead embryos (Mauldin, 2002). For candling, a table candler can be used, which is faster or a spot candler which is slower but more accurate. The process where candled eggs regarded as non-viable are broken, and opened to determine fertility and development of the embryo is termed egg breakout (Mauldin, 2002). For embryo mortality, classification is often expressed as early, mid and late dead (Wilson, 1995). In the first week of incubation, embryo death is classified as early dead, and characterized by a network of blood vessels with possible adherence to the side of the egg. In the second week, dead embryos exhibit a hard beak and an egg tooth, while late dead embryo (third week incubation) manifest full feather (Wilson, 1995).

2.12 *In Vitro* Sperm Penetration Assay

Bramwell and Howarth (1992a) developed a method in which they were able to quantitatively analyze the sperm-egg interaction *in* v*itro*. Perivitelline layer used in these studies was removed from a recently ovulated ovum prior to their use in the *in*

vitro assay. Following co-incubation with spermatozoa, the perivitelline layer is stained with a Schiffs reagent, after which it takes on a dark maroon color. As light passes through the clear distinct holes digested in the perivitelline layer by sperm which have undergone an acrosome reaction, the holes are then able to be easily counted under a light microscope. This *in* v*itro* method of quantifying sperm penetration has since been successfully utilized as an accurate and useful measure of activity of spermatozoa where fertilization is localized (Bramwell and Howarth, 1992b).

The primary disadvantage of the *in* v*itro* method of analyzing sperm penetration mentioned above, is that it requires hens to be killed in order to retrieve the recently ovulated ova from which the perivitelline layer is removed. Because hens must be killed at approximately 20 minutes post oviposition, these hens must be individually caged in order to identify the exact time in which a hen lays her egg (Bramwell and Howarth, 1992a). This limits the types of research for which this method can be utilized. For economic reasons, it limits its usefulness on a large scale basis; from a practical standpoint, essentially preventing its use in commercial breeder flocks. Also, having to kill at least one hen to obtain each perivitelline layer makes studies with time as one of the variables extremely difficult and costly to run. It would also prohibit observations to be made on individual hen overtime. However, this method allows for comparisons to be made between sperm from individual males on the perivitelline layer from a single female. This type of test removes all female storage, transport and other physiological variation and only measures different groups of semen in their physiological capability to bind and penetrate the perivitelline layer (Bramwell and Howarth, 1992a).

2.13. Care of Hatching Eggs

Only clean eggs without cracks and minimum 52g from flock of at least 24 week of age are recommended for incubation. In setting eggs less than 52g in weight should be avoided to prevent good quality chicks. While weight of egg and age of breeder influence chick quality, growth of the female at the beginning of lay also affect quality (Wilson, 1991). Rate of lay in breeding flock usually starts rapidly and if feed intake is not proportionately increased with increased in egg production, nutrients transfer of the egg is hampered resulting in poorer chick's viability (Wilson, 1991). On the other end, rate of hatch is lower with eggs that are too large, and eggs weight in excess of

70g should be removed. If egg weight during lay is to be controlled and extra-large eggs are to be avoided at the culmination of production, the facts below need to be noted:

- Weight of pullet at 5% lay: Pullet weight at onset of lay largely influence average weight of eggs. Overweight pullets at commencement of lay will produce bigger eggs throughout the life of the flock.
- Limiting oil content of feed or substituting it with saturated fats. Increasing oil induces increase in feed intake, and associated increase in weight of eggs.
- Lowering amino acids content of layers diet: This can help reduce egg weight, however, with simultaneous reduction in rate of lay. Altering amino acids levels is thus discouraged during laying.
- Intake of energy: From 40 weeks of old, a moderate reduction of around 50 Kcal in feed energy level could be used to stabilize weight of egg, as long as those changes in feed composition do not lead to under consumption.
- Temperature: Lower pen environmental temperature could lead to increased intake of feed and therefore increases the weight of egg, it should be avoided.

2.13.1 Eggs Collection

Cages are better fitted with an ejection system to prevent the birds polluting the nest by night-time. Cages necessarily need to always be set aside clean. Droppings and damaged eggs must be regularly removed. The nesting material needed to be unsoiled and without mold and be reformed frequently. In automated cage, plastic bottoms needed to be washed off on a regular basis.

Collection of eggs for incubation must be carried out for at least 3-4 times in a day. The rate can be increased in hot or cold weather to avoid embryo development to start developing at temperature beyond 22°C (Reis *et al.*, 1997). Overtime fluctuations in temperature may result to early embryo death that could be mistaken for infertility (Antwi, 1993). Egg collecting crates should have holes for faeca impurity, and remains from external part of egg shell, in order not to soil or pollute other eggs. The crates must be scrubbed, cleaned and sanitised beforehand. Re-use of previously used cardboard trays is not recommended.

Proper hygiene of the hands is essential before handling eggs. The use of a sanitizing solution after washing the hand is advised and this should be done frequently during egg collection and after other subsequent tasks.

2.13.2 Floor Eggs

As a precaution, hatching eggs should not contain floor eggs. However, economic constraints may require that clean floor eggs collected quickly can be incubated in a dedicated setter, if disinfected immediately after lay. Under no circumstance should dirty eggs be stored near clean hatching eggs, they must be eliminated. As a general observation, hatching is poor with floor eggs (Branett *et al.*, 2004). Improve management practice can reduce the number of floor eggs.

2.13.3 Eggs Sanitation

Hatching eggs should be subjected to disinfection upon collections advisably within 3 hours after collection. Several methods could be used, however formalin remains the best fumigant where regulation allow it usage.

Where formalin use is prohibited, methods such as fumigation with chlorine, hydrogen peroxide, ammonium compound, exposure to ozone or UV rays, or the use of disinfection solution remain effective (Berrang *et al.,*1997).

2.13.4 Fumigation

For fumigation to be effective, the following rules must be adhered to:

- Eggs should be disinfected instantly after lay, while still hot.

- Ambient temperature of 24°C and 80% (RH) relative humidity.

- A mixture of formalin 30ml of 40% and 20g permanganate is ideal per cubic meter of space or 10g of formaldehyde powder.

- Fumigation should be carried out within 20 minutes and elimination of the formaldehyde gas from the chamber done in 10 minutes (Braun *et al*., 2011).

2.13.5 Disinfection by Spraying

On the farm, an acceptable hatching egg sanitizer must be used to spray the eggs on the farm and each tier or row needs to be sanitised before putting the next tray above the last tray. For proper decontamination by spraying, eggs ought to be directly put in setter trays and appropriate spraying method with satisfactory droplets is required. When not correctly carried out, fumigation by spraying has several disadvantages in which *In vivo* contamination occur as a result of too much spraying or the droplet

size is undue. Eggs that are excessively wet get cool rapidly and absorb egg shell germs via the pores. These are causes of eggs exploders in setters and bad chick's quality at day old (Brake and Sheldon, 1990).

2.13.6 Eggs Storage

Before placement in the storage rooms, eggs should be allowed to cool for 1- 2hours at a room temperature between 16˚and 18˚C, based on storage duration. In regions where ambient temperatures surpass 22˚C, a room storage fitted with air conditioning would be an appropriate investment. For brief period of storage, ambient condition of 18 ˚C and 80% RH are ideal, for longer duration (>6days), 15 ˚C is required (Reis *et al*., 1997). Low temperature on the shell of egg is a risk when eggs warm up after storage, and must be avoided as moisture and gases can penetrate the egg shell (Brake *et al*., 1997). As embryo develop, gaseous exchange is normally expected, however excessive moisture loss from within the egg will lead to a reduction in hatchability and viability of chicks (Lapao *et al*., 1999). Another recommendation for storage rooms is automatic humidifier levels. Where this is unavailable, water can be sprayed frequently on the concrete floors of the room.

In addition, the storage room should be insulated, dust free and preferably tiled to ease cleaning and fumigation. Eggs in storage should be placed with the narrow end down when stored for a short period. For longer periods exceeding 7days, placement with the broad end down reduces loss in hatchability due to age. Never should hatching eggs be stacked directly on the floor, but on slates which can be wooden or plastic

CHAPTER THREE

MATERIALS AND METHODS

3.1 Brief Description of Study Location

Animal Physiology Laboratory, Department of Animal Science and poultry Unit of the Teaching and Research Farm were for this experiment. Both were located at University of Ibadan, Ibadan, Nigeria, which was 100 miles away from the Atlantic coast in the Southwest region of Nigeria. It is a rain forest zone about 200m above sea level at 7˚27^{1}192^{11}N,3˚53^{1} 45.9^{11} E.

3.2 Experimental Animals

Marshall broiler breeder chickens sourced from a reputable farm in Ibadan were used for the study. The breeder hens were kept in cage, while the cock were raised on the deep litter. The animal were served commercial breeders ration (Hybrid Feed brand). Fifteen cocks were trained for collection of semen through belly massage technique of Adebisi and Ewuola (2019a). That training was carried out for two weeks and for each cock, semen was examined for colour, sperm motility, sperm liveability and concentration of sperm cells.

3.3 STUDY 1: EFFECTS OF SEMEN DOSES AND OVIDUCTAL SPERMATOZOA AGE ON FERTILITY AND EMBRYONIC DEATH IN BROILER BREEDER CHICKENS

3.3.1 Experimental Layout

Fifteen cocks (3.5±0.45kg) and one hundred and twenty hens (2.8±0.34 kg) thirty two weeks old were used for the experiment. Randomised method were used to distribute the hens using 5 treatments and 4 replicates (6 chickens / replicate). Fresh Semen collected from 10 cocks out of fifteen, was pooled together and carefully inseminated into hens at doses of 0.02mL (T1), 0.04mL (T2), 0.06mL (T3), and 0.08mL (T4), for two consecutive days. A part of the semen pooled before insemination was subjected

to semen assessment. Group 5 (T5) Hens were naturally mated, one cock to 4 hen at ratio 1:4, for two consecutive days. Each hen was mounted once for each of the two days and the mating was supervised to ensure it was successful.

3.3.2 Collection of Semen from the Cocks

The cocks were restrained and semen extracted using dorsal-abdominal massage technique as described by Adebisi (2018). Two people were required for the collection. Each cock is restrained by holding firm the two thighs together with the left hand and resting the chest on flat surface. The dorsal part of the abdomen was stroked with rapid massages around the vent and thigh for stimulation, at the completion of the massages the cock responded by the erection of the phallus. The right hand was then used to push backward the tail feather and the thumb and forefinger was placed at the vent to get out semen from the bulbous duct. Another person help in holding the tube for collection as the semen flowed unto it. Semen indicating poor quality as a result of contamination with blood and faeces was discarded. The semen was pooled together and gently mixed before semen evaluations.

3.3.3 Semen Evaluation

3.3.3.1 Colour: Visual assessment was utilized. Good cock semen should not contain any contaminant and the colour should be creamy white (Ewuola and Egbunike, 2010).

3.3.3.2 Progressive Spermatozoa Motility: This was determine by putting a droplet of fresh semen in a clean sterile slide and adding a droplet of warmed sodium citrate. A glass slip was used to cover the glass slide and viewed at an x400 magnification. Progressive movement was determined by a subjective score ranging between 0 and 100% (Ewuola and Egbunike, 2010).

3.3.3.3 Sperm Liveability: Live spermatozoa (%) was determined by mixing semen (a drop) with Eosin-Nigrosin stain (a drop) and then smeared on the slide, and allowing the slide to air-dry. Afterward, the slide was observed under the microscope at ×400 magnification. The dead spermatozoa absorbed the stain. Liveability of spermatozoa can be express by calculating the number of live sperm cells as a percentage (%) of total sperm cells examined (Ewuola and Egbunike, 2010).

3.3.3.4 Sperm Concentration. The concentration of sperm cells was determined by visual count of spermatozoa using Neubauer haemocytometer. 20µL of semen was

diluted with 200µL of 0.9% saline to fix the cells. The mixed semen was then charged on each of the two chambers of the haemocytometer using a micro pipette. The charged haemocytometer was viewed under light microscope at magnification of x400 (Ewuola and Egbunike, 2010). Sperm cell concentration per mL was calculated by means of the formula below:

C = n x d x 50000……………………………………………………….(1)

Where letter C = spermatozoa concentration

n = sperm cells number counted in five diagonal squares

d = dilution factor

50,000 a constant resulting from five squares area counted and haemocytometer depth

3.3.4 Deposition of Semen into the Hens Oviduct

To commence insemination, the hens cloaca was everted to allow for spermatozoa deposition into the oviduct of the hen using graduated tuberculin syringe with an attached glass rod (Adebisi, 2018). The collection of the semen from the cocks and deposition into all the hens were done around one hour. This was carried out in the evening to ensure that there is no egg inside the oviduct during insemination, to ensure fertilization took place and also to avoid semen wastage. The volume administered to each hens are stated in the experimental layout.

3.3.5 Egg Collection and Storage

Egg was first collected a day after the second insemination and this was done daily for each treatment. The eggs were marked and kept on crates at environmental condition of 25˚C to 27˚C and 70% - 85% relative humidity as measured by thermo-hygrometer (Rexson product, India New Delhi) for 7days before eggs incubation.

3.3.6 Egg Incubation and Candling

The eggs were incubated every week for a period of four weeks. Prior to setting, the eggs were fumigated in an enclosed room with a mixture of formalin and potassium permanganate for 40mins at the hatchery. 37.5˚C and 85% was set as incubation temperature and humidity for chicken eggs. Candling of eggs was done on the 18th day, during eggs candling, all clear eggs were removed, fertile eggs with developing embryos were transferred to the hatcher machine. Temperature of the hatcher was 36˚C and a

humidity of 90% was used. On 21st day, eggs complete incubation, chicks that were hatched and unhatched eggs were all removed, while broken out was done to ascertain any infertile eggs and fertile egg with evidence of dead embryos (Ewuola and Adebisi 2019a).

3.3.7 Assessment of Fertility, Embryonic Death and Hatched Eggs

Embryo deaths was classified as early which occurred within the first week of incubation, 8th - 18th days as mid and 18th - 21st days of incubation is late embryo death as characterized by embryonic anatomy and size (Beaumont *et al*., 2002). Fertile eggs were considered as the sum total of hatched chicks and dead embryos. Hatch of set eggs (%), dead embryos (%) and fertility (%), at each stage were expressed below by their corresponding formula.

Fertility (%) = 100 X fertile eggs number / set eggs number ………………………. (2)

Dead embryo (%) = 100 X dead embryo number / fertile eggs number ……………(3)

Hatch of set eggs (%) = 100 X hatched chicks number / eggs set number…………...(4)

3.3.8 Determining the Duration of Fertile Period and Oviductal Sperm Age

Efficient length of fertile period and maximum duration of fertile period are both used for duration of fertile period assessment. The numbers of days post- insemination during which maximum production of fertile eggs takes place is considered as the effective duration of fertile period, while the length of day it takes to record zero fertility post - insemination is determined as maximum duration of fertile period (Liu *et al*., 2008). Oviductal sperm age is the period of time where live spermatozoa inhabit the oviduct (Tabatabaei *et al*., 2009).

3.3.9 Statistical Analysis

Data was subjected to log transformation to normalise data. Transformed data were analysed using the general linear model and where significant were separated using Duncan's multiple range test ($P < 0.05$). Data for fertility against time post- insemination was fitted to linear regression model.

3.4 STUDY 2: EFFECTS OF INSEMINATION FREQUENCY ON FERTILITY, EMBRYONIC, DEATH AND HATCHABILITY IN BROILER BREEDER CHICKENS

3.4.1 Layout of the Experiment

One hundred breeder broiler hens and twelve cocks (38 week of age) were used for the study. The females were randomly allotted to four treatments replicated five times, in a completely randomised design. Harvested semen from the cocks was pooled and some part was used to assess for progressive motility, liveability, and concentration of spermatozoa as earlier explained in study one. Undiluted semen was administered at 0.02ml/bird, with insemination frequency as the basis for the treatments. Intervals of insemination were 3 days (T1), 6 days (T2), 9 days (T3) and 12 days (T4). Insemination was carried out for 15 weeks.

3.4.2 Assessment of Fertility, Hatch Parameters and Embryo Death

Collections of eggs and storage, incubation and candling were carried out as done in study 1. The eggs that were not hatched (unhatched) were broken-out and visually checked to ascertain the amount of infertile eggs in each treatment. Embryonic deaths were in three stages, early deaths occurred within the first week (1st - 7th) days of incubation, mid (8th - 18th) day and late (18th - 21st) day as characterised by embryo anatomical features and size (Beaumont *et al*., 2002). Fertile eggs were considered as the sum total of hatched chicks and dead embryos. Fertility, dead embryo at each stage and hatch parameters calculation were express as shown in study one above.

Fertile eggs hatch (%) = 100 X chicks hatched number / eggs fertile number…… (5)

3.4.3 Determination of Chicks Quality Features

Determination of chicks features quality was carried out on 21st day of incubation at the hatchery. Chicks from each treatment were assessed for qualities clean and dry feather, colour, clear and sharp eyes, deformity free and sealed abdominal navel part (Decuypere *et al*., 2001). The beak of chicks must be close and not bend so also the firm and straight toes is expected to be seen. Chick alertness and response to sound was characterised as good quality (Tona *et al*., 2004).

Chicks quality (%) = Number of quality chicks x 100……………..(6)

3.4.4 Data Collection and Statistical Analysis

Percentage data were subjected to transformation before analysis. Transformed data were analysed using the general linear model of SAS (2003). Duncans multiple range test was used to separate significant main effect means.

3.5 STUDY 3: EFFECTS OF DEXTROSE AND NORMAL SALINE AS DILUENTS ON SEMEN QUALITY, FERTILITY AND HATCHABILITY BROILER BREEDER CHICKENS

Experiment one: ***In Vitro*** **Laboratory Experiment to Determine the Effect of Dextrose Saline and Normal Saline (0.9%) as Semen Diluents under Room Temperature (27.1**°C **to 27.8**°C)

3.5.1 Experimental Layout

In this experiment, semen from seven breeding broiler cocks were pooled homogenized by mixing and divided into 4 parts. The first three portions were diluted at ratio 1:1 with 0.9% Normal saline, 1% dextrose saline and modified Ringer's solution, respectively, while the 4th portion remained undiluted. Diluents component of the three above is presented in Table 3.1 below. (0.9%) Normal saline, Contains 9g of sodium chloride in a liter of sterile water, while 1% dextrose saline was reconstituted from 5% Dextrose in 0.9% sodium chloride solution. Dextrose saline (5% / 0.9%) has an osmolarity of 560m Osm/L, a value higher than the osmolarity of sperm cells. Reconstitution was done by diluting four parts of 0.9% saline with one part of 5% dextrose saline to give 1% dextrose in 0.9% saline solution. Osmolarity of reconstituted 1% dextrose saline was 363mOsm/L.

$$\text{Osmolarity (mOsm/L)} = \frac{\text{Weight of solute in g/L}}{\text{Molecular weight of solute}} \times \text{Number of ions in solute} \times 1000$$

3.5.2 *In vitro* Evaluation of Semen

In vitro evaluation was carried out hourly at room temperature (27.1°C to 27.8°C). This assessment was carried out till sperm motility dropped below 50% (Adebisi, 2018). Parameters assessed were:

3.5.2.1 Progressive Spermatozoa Motility: This was done by putting raw semen, a drop on a microbial free glass slide and immediately adding a drop of slightly warmed

Table 3.1: Constituent of Diluents

Composition (g/100ml distilled water)	Modified Ringer's solution	Normal Saline	Dextrose Saline
NaCl	0.68	0.90	0.90
KCl	0.17	_	_
$CaCl_2$	0.06	_	_
$MgSO_4$	0.03	_	_
$NaHCO_3$	0.24	_	_
Dextrose	_	_	1.00
P^H	6.74	6.79	6.70
Osmolarity (mOsm/L)	333	310	360

Tabatabaei, 2010

sodium citrate of the same temperature. The prepared slide was gently covered with a slip and viewed under the microscope (x400 magnification). A subjective score between 0 and 100 % was used to rate progressive movement of the sperm cells (Ewuola and Egbunike, 2010).

3.5.2.2 Integrity of Plasma Membrane: The hypo-osmotic swelling test procedure was carried out as explained by Jeyendran *et al.* (1984). Briefly, 1g of sodium citrate was used and dissolved inside 100ml of distilled water to produce a hypo- 0smolarity solution (100mOSm). 0.1ml of semen was added with hypo - osmotic solution of 1mL. Mixture of the two was heated putting it inside a water bath for 30minutes at temperature of 37°C. Spermatozoa were viewed for curved tails, indicating swelling of the cells, under a microscope (x400 magnification). Plasma membrane integrity was determined as the number of spermatozoa with curved tails as a percentage of total spermatozoa viewed.

3.5.2.3 Sperm Liveability: Live spermatozoa was assessed by putting a drop of semen on a glass slide and staining with Eosin-Negrosin (one drop). Semen samples were mixed and smeared gently on another clean slide. Excess stains on the slide was blown off with mouth after which it was left on the shelf to air dry. After air-drying the slides, they were all observed under the microscope at ×400 magnification. Eosin – Negrosin stains was absorbed by dead spermatozoa. The live cells were determined by deducting the dead cells from the estimated total cells under view. Liveability was calculated by expressing the live cells as a percentage of the estimated total cells viewed Ewuola and Egbunike, (2010).

Experiment two: Effect of Dextrose and Normal Saline as Semen Diluents on Fertility and Hatchability of Broiler Breeder Chickens

3.5.3 Layout of Experimental

Seven Marshal breeder cocks and eighty broiler breeder hens were used in this experiment. Hens were allotted randomly to five treatments of 4 replicates and 4 hens each in a completely randomised design.

3.5.4 Procedure for Collection of Semen, Dilution and Insemination

Fresh semen collection was done by collecting semen from the cocks which were pooled together to remove variability and separated into four parts. The first three parts

were diluted with 1% dextrose saline, normal saline and modified Ringer's solution in ratio 1:1 respectively (Table 3.2), while the last part remained undiluted. Insemination was concluded by everting the cloaca of each hens and depositing semen inside the oviduct. Collection of semen as well as dilution and insemination of all the chickens were done in about 1 hour and it was carried out in the evening to reduce stress and wastage of semen. The insemination was carried out for two days successively. The semen inseminated were assessed for sperm concentration, motility and liveability following standard methods.

3.5.5 Egg Collection, Storage and Incubation

Each day, eggs were collected, marked and stored at ambient condition of 25°C to 27°C and 70% - 85% R H for a week. Eggs were incubated weekly for a period of 4weeks.

3.5.6 Determination Fertility and Hatchability of Eggs

On the 18th day of eggs incubation, the candling of eggs was carried out to separate "clear" eggs. All unhatched eggs at day 21 were broken out and assessed as described in study one. Total fertile eggs was the sum total of dead embryo and all the hatched chicks.

Percentage fertility and hatchability were calculated below:

Fertility (%) = 100 X fertile eggs number / set eggs number………………………(2)

Hatchability (%) = 100 X chicks hatched number / set eggs number………………(5)

3.5.7 Statistical Analysis

Data collected was put through analysis of variance by using General linear Model (SAS, 2003). Duncan's multiple range test of the same software was used to separate significant main effect of means. *In vitro* parameters against assessment time and fertility against time post- insemination were fitted to a linear regression model.

Table 3.2: Layout Indicating Diluent and Semen Volume Inseminated

(M. R) - Modified Ringer's

Treatment (Diluents)	Semen to Diluent Ratio	Semen Volume (mL)
Normal saline	1:1	0.02
M. R. solution	1:1	0.02
Dextrose saline	1:1	0.02
Raw semen	1:0	0.01
Raw semen	1:0	0.02

3.6 STUDY 4: EFFECTS OF DILUTION OF SEMEN AND FREQUENCY OF INSEMINATION ON FERTILITY, EMBRYONIC DEATH, HATCHABILITY AND OVIDUCTAL SPERM COUNTS IN BROILER BREEDER CHICKENS

3.6.1 Experimental Layout

Eighty one breeder hens were randomly divided into 9 treatments, of 3 replicates and 3 hens/replicate, while semen from seven cocks were pooled for insemination. Study design was a 3 x 3 factorial arrangement in a completely randomised design.

Factor 1 – Dilution ratio; raw semen (1:0), 1:1 and 1:2)

Factor 2 – insemination Frequency of (3, 6, and 9 days)

3.6.2 Assessment of Fertility, Embryonic Death and Parameters of Hatch

The eggs were collected daily, stored for incubation and insemination of hens was carried out for a period of 10 weeks. Candling and break- out were done as explained in study one above. Egg Fertility, embryo mortality, hatch parameters was carried out as described in study 1.

3.6.3 Statistical Analysis

Data were analysed by using descriptive statistics and two – way analysis of variance by means of general linear Model of SAS (2003). Duncans multiple range test was used in separating means.

3.6.4 Determination of Spermatozoa Number in Different Oviductal Segments

At the end of the study period, three hens per treatment were sacrificed for Oviductal sperm count by the wash-out method (Brillard and Bakst, 1990). Tween 20 was used to replace Triton x-100 in this study. After sacrifice, the entire oviduct was removed and separated into 3 segments. Infundibulum, magnum /isthmus and uterus /vagina. The infundibulum was ligated at the base region using meter rule and thread to separate segment into 1cm distal towards infundibulum – magnum section to form segment number one. Second ligation was below to the proximal part of uterine gland while the third opening was ligated at distal part of vagina to mark second and third sections. Each segment was inoculated using 3.5 ml of the Hank's Balanced Salt Solution (HBSS) and 0.05 percent Tween twenty. The HBSS composition is presented in Table 3.3. The solution was carefully rubbed throughout and within the mucosal pleats. Each of the sections was cut open to retrieve the fluid that was injected and also the volume

was recovered and noted. Spermatozoa recovered was determined by haemocytometric count with the aid of counter (10 replicate counts was taken for each washout).

Table 3.3: Chemical Components of Hank's Balanced Salt solution

Composition	Gram/liter of water
Glucose	1.00
Na_2HPO_4	0.05
KH_2PO_4	0.06
$NaHCO_3$	0.35
$MgSO_4$	0.10
$CaCl_2$	0.18
NaCl	8.00
KCl	0.4

Source - Hi-Media Laboratory India (2011)

The concentration of sperm cell for each volume was determined as described by Adebisi and Ewuola (2019a) by the formula below:

C = n x d x 50000…………………………………(1)

Where C indicate = sperm concentration

n = sperm cell number counted in 5 diagonal squares (Ewuola and Egbunike, 2010).

d = dilution factor (volume of wash-out and diluent mixed)

50,000 is constant resulting from five square area counted and haemocytometer depth

3.6.5 Spermatozoa Number at the Tubular Storage of the Uterovaginal Section

In modification to the method described by Brillard and Bakst (1990), cuts were made longitudinally along the uterus and vagina (2cm proximally and 2cm distally to the Uterovaginal juntions). Mucosa were separated from the section. Surface folds were gently scraped to separate from the muscularis mucosa. Scraped folds pulverized in 4ml HBSS and filtered through muslin cloth. To recover spermatozoa inside the SST (Adebisi, 2018), the filtrate (0.1 mL) was diluted in HBSS (1.9 mL) to give a dilution factor of 20 (Adebisi, 2018). Sperm cells per ml of filtrate were counted using haemocytometer and calculated using the formula; described in section 3.6.4.

3.6.6 Statistical Analysis

Data were put through analysis of variance using General linear Model SAS (2003) and significant means were separated using Duncan's multiple range test

CHAPTER FOUR

RESULTS

4.1 STUDY 1: EFFECTS SPERMATOZOA NUMBER AND OVIDUCTAL SPERM CONDITION ON FERTILITY, EMBRYONIC DEATH AND HATCHABILITY IN BROILER BREEDER CHICKENS

4.1.1 Semen characteristics of cock Pooled for insemination

Pooled semen characteristics used for this study are shown in Table 4.1. Mean sperm concentration observed was 0.28±0.13 (x10^9 /ml). Sperm cell liveability (%) and motility values were 96.8±3.7% and 90.0±0.0%, respectively.

4.1.2 Effect of Semen Dose on Fertility in Broiler Breeder Hens

The effect of semen insemination doses on fertility of sperm cells in broiler breeder hens are presented in Table 4.2, The First week post - insemination fertility (%) did not differ among the inseminated groups. With values that ranged from 94.29±1.91% (T1) to 97.11±2.88% (T4) but significantly higher than T_5 (76.89±2.95%) which is the naturally mated group. However, at 14 days post-insemination, fertility did not differ among the treatments. The value ranged from 41.89±8.52% (T5) to 66.60±9.01% (T_1). At 21 days, similar fertility values were observed in T_1 (15.50±6.32), T_2 (12.89±4.50%), T_3 (9.37±4.40%), T_4 (10.42±3.79%) and T_5 (6.22±2.17%). However, at day 28 post - insemination, fertility decline sharply across all groups and all eggs were infertile in all the treatment groups.

Table 4.1: Characteristics of Pooled Semen Used for Artificial Insemination

Parameters	Mean values (± Standard deviation)	Range
Sperm concentration (x 10^9 cells/mL)	0.28±0.13	0.27 – 0.30
Liveability (%)	96.80±3.69	90.00 – 100.00
Sperm motility (%)	90.00±0.00	90.00 – 90.00

Table 4.2: Egg Fertility (%) from Broiler Breeder Hens Inseminated With Varied Semen Doses for Four Weeks

Weeks post - insemination	Dose of semen inseminated (mL)				
	0.02 (T1)	**0.04 (T2)**	**0.06 (T3)**	**0.08 (T4)**	**N.M. (T5)**
1	94.29 ± 1.91^{a}	94.53 ± 3.23^{a}	95.17 ± 1.91^{a}	97.11 ± 2.88^{a}	76.89 ± 2.95^{b}
2	66.60±9.01	56.03±4.41	55.17±7.54	47.01±9.32	41.89±8.52
3	15.50±6.32	12.89±4.50	9.37±4.40	10.42±3.79	6.22±2.17
4	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

[ab] Means of treatments along a row with different superscripts differ ($p<0.05$). (Mean±SE).

N.M.: Natural mating

4.1.3 Effect of Fertile Length and Oviductal Sperm Condition on Eggs Fertility in Broiler Breeder Chickens

Fertile length and oviductal sperm age effect on egg fertility in broiler breeder hens is shown in Figure 4.1. The mean value observed at maximum fertility of 95.26% for the first 7days post-insemination. Fertility dropped steadily and significantly on daily basis to 25% at day 14, after which it remained below this figure till it finally reached 0% at day 22.

The effect of fertile period duration and oviductal spermatozoa age on egg fertility in hens from naturally mated group is shown in Figure 4.2. Fertility reached a maximum of 76% up till day 7 and dropped to 60% between day 9 and day10 with a sharp decline to about 10% on day 11. There was an increase to 39% at day 14, and a steady decline to 0% was observed at day 22.

4.1.4 Predicting Fertility (%) Using Linear Regression of Days Post - Insemination in Broiler Breeder Chickens

The relationship between fertility (%) and days of post - insemination in broiler breeder hens is presented in Figure 4.3. There was an inverse linear relationship observed between fertility and period post insemination. The R^2 value (0.87) indicated that a strong inverse relationship existed between fertility and post insemination period with subsequent decline in fertility as post-insemination day increases.

4.1.5 Effect of Dose of Semen Inseminated on Fertility, Embryonic death and Hatch of Egg Set at First Week Post – Insemination in Female Broiler Breeders.

The influence of sperm number (insemination dose) on embryonic death and hatch able egg set in broiler breeder at first week post artificial insemination is shown in Table 4.3. Fertility was not at variance among the inseminated groups, but these values were significantly ($p<0.05$) higher (94.29% to 95.17%) than the naturally mated group (76.89%). Early, mid, late and total embryo mortality were similar across the groups. The percentage of eggs hatched from the number of set eggs was significantly (P<005) higher at T4 (90.49±3.53%) than T5 (75.10±3.14%). However, they were both similar to other groups.

4.1.6 Effect of Dose of Semen Inseminated on Fertility, Embryonic Death and Hatch of Eggs Set at Second Week Post Insemination in Boiler Breeders Hens

Insemination dose effect at two weeks post - insemination is presented in Table 4.4, No significant difference was observed for fertility, early, mid and late embryonic mortality as well as total embryonic mortality and hatch of eggs set. Fertility values ranged from 41.89% (T_5) to 66.60% (T_1) while hatch of set eggs ranged from 37.04% (T5) to 58.82% (T1).

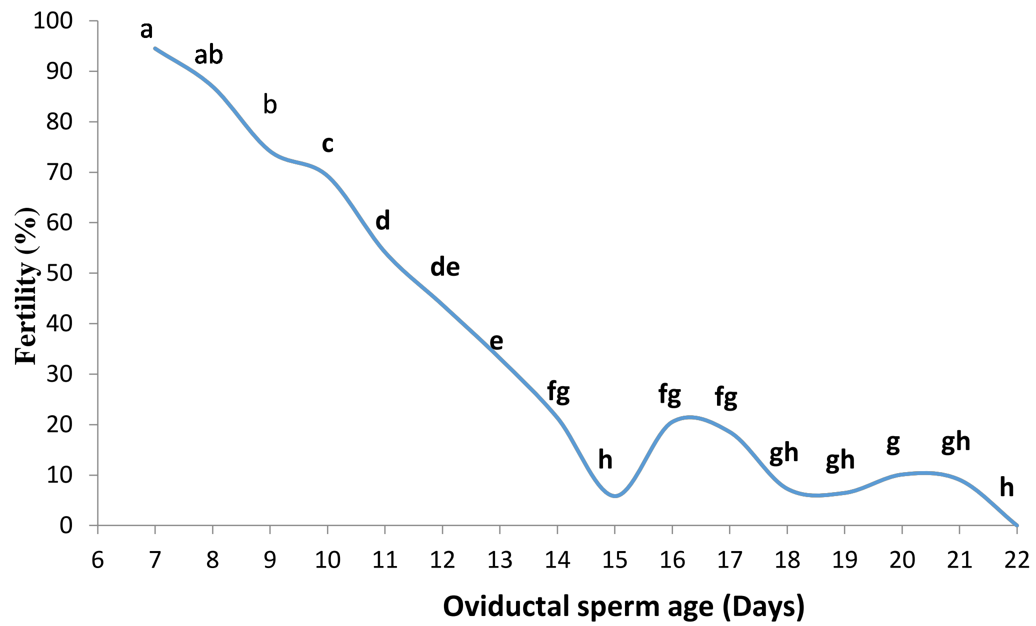

Figure 4.1: Duration of Fertile Length and the Effect of Oviductal Sperm Age in Days on Egg Fertility in Inseminated Broiler Breeder Hens

a,b,c,d,e,f,g,h - Means with different letters are significantly (p<0.5) different

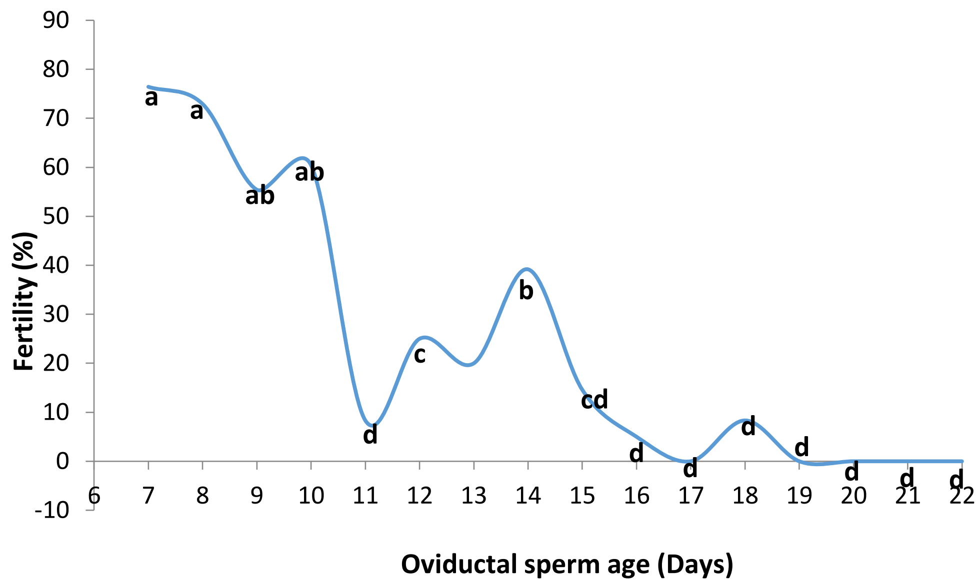

Figure 4.2: Duration of Fertile Length and the effect of Oviductal Sperm Age in Days on Egg Fertility from Naturally Mated Broiler Breeder Hens

a,b,c,d, - Means with different letters are significantly ($p<0.5$) different

4.1.7 Effect of Semen Dose on Fertility, Embryonic Death and Hatch of Eggs Set From Broiler Breeder Three Weeks Post – Insemination

Semen insemination dose effect at the 3rd week post insemination is presented in Table 4.5. It was observed that the effect of semen insemination doses at third week post-insemination on fertility, embryo death and hatch of set eggs was not at variance among the treatments. Values for fertility ranged from 6.22% (T5) to 15.50% (T1), while hatch of set eggs ranged from 5.22% (T5) to 15.50% (T1).

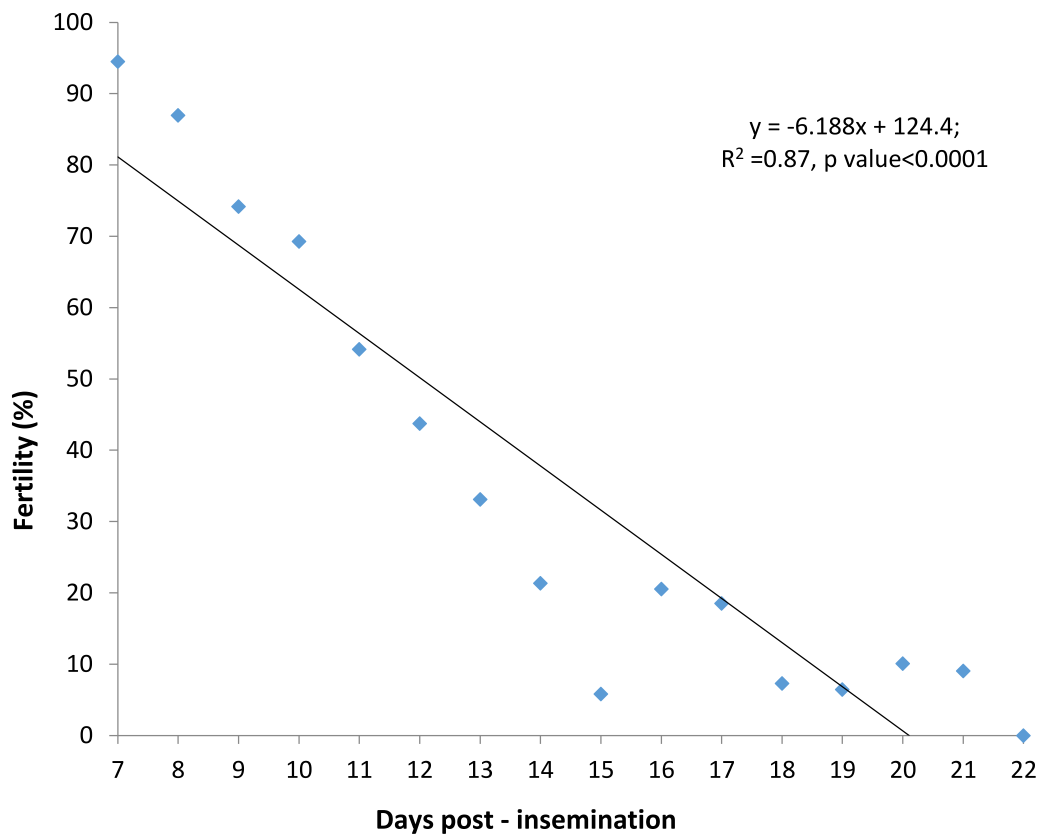

Figure 4.3: Linear Regression of Fertility against Days Post-Insemination in Broiler Breeder Hens

Table 4.3: Semen Dose Effect on Fertility, Embryonic Death and Hatchability ofEggs Laid From Female Broiler Breeders in the First Week Post-Insemination with Different Doses

Semen inseminated	Fertility (%)	Embryonic mortality (%)				Hatch of set eggs (%)
		Early	Mid	Late	Total	
0.02mL (T1)	94.29 ± 1.91^{a}	0.00±0.00	0.00±0.00	8.89±3.43	8.89±3.43	85.93 ± 3.85^{ab}
0.04mL (T2)	94.53 ± 3.23^{a}	1.90±1.11	0.00±0.00	4.93±3.63	6.83±2.86	88.03 ± 3.82^{ab}
0.06mL (T3)	95.17 ± 1.91^{a}	2.13±1.24	0.00±0.00	10.05±4.10	12.19±4.93	83.81 ± 6.24^{ab}
0.08mL (T4)	97.11 ± 1.91^{a}	1.08±1.09	0.00±0.00	5.65±3.48	6.74±3.04	90.49 ± 3.53^{a}
N. M. (T5)	76.89 ± 2.95^{b}	1.13±1.14	0.00±0.00	1.13±1.14	2.27±2.27	75.10 ± 3.14^{b}

(a, b) – Means in a column with different superscripts differs (P<0.05)

N. M. - Natural mating, (Mean ± SE) - Standard error;

Table 4.4: Semen Doses Effect on Fertility Percentage, Embryo Death and Hatchability of Eggs laid from Female Broiler Breeders on Second Week Post-Insemination with Different Doses

Semen inseminated	Fertility (%)	Embryonic mortality (%)				Hatch of set eggs (%)
		Early	Mid	Late	Total	
0.02mL (T1)	66.60±9.01	0.00±0.00	8.33±8.33	5.84±2.11	14.18±6.43	58.82±10.7
0.04mL (T2)	56.03±4.40	1.90±1.92	0.00±0.00	10.47±4.80	12.39±6.55	49.21±5.55
0.06mL (T3)	55.17±7.54	0.00±0.00	5.02±2.98	4.59±2.10	9.62±3.73	49.88±6.97
0.08mL (T4)	47.01±9.32	0.00±0.00	6.25±6.25	5.36±5.36	11.61±6.74	42.56±9.74
N. M. (T5)	41.89±8.52	3.33±3.33	1.79±1.79	10.00±7.93	15.12±7.56	37.04±8.89

(Mean ± SE) SE- Standard error; N. M. - Natural mating

Table 4.5: Semen Doses Effect on Fertility, Embryo Death and Hatchability of Eggs Laid from Female Broiler Breeders on Third Week Post-Insemination with Different Dose

Semen inseminated	percentage Fertility	Embryonic mortality (%)				Hatch of eggs set (%)
		Early	Mid	Late	Total	
0.02mL (T1)	15.50±6.32	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	15.50±6.33
0.04mL (T2)	12.89±4.50	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	12.89±4.50
0.06mL (T3)	9.37±4.40	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	9.37±4.40
0.08mL (T4)	10.42±3.79	0.00±0.00	12.50±12.50	0.00±0.00	12.50±12.50	9.11±4.00
N. M. (T5)	6.22±2.17	0.00±0.00	0.00±0.00	12.50±12.50	12.50±12.50	5.22±2.13

N. M. – Natural mating, (Mean ± SE)- Standard Error

4.2 STUDY 2: INFLUENCE OF FREQUENCY OF ARTIFICIAL INSEMINATION ON FERTILITY, EMBRYONIC DEATH AND HATCHABILITY IN BROILER BREEDER CHICKENS

4.2.1 Pooled Semen Inseminated for Fifteen Weeks

Pooled semen inseminated for a period of fifteen weeks is shown in Table 4.6. The mean value of sperm concentration observed was 0.48±0.17 x10^9/ml. The mean values of sperm liveability and progressive spermatozoa motility were 98.6±9.0% and 93.1±2.5%, respectively.

4.2.2 Egg Fertility, Embryonic Death, Hatchability and Chicks Quality of Broiler Breeder Hens Inseminated with 0.02mL Undiluted Semen at Different Insemination Frequency

The effect of frequency of insemination on fertility, embryo mortality and chick quality of broiler breeder hens is presented in Table 4.7. At 3 days insemination frequency fertility (80.36±1.76%) was significantly (P<0.05) higher than 6 days (74.28±1.75%) and 12 days (67.79±2.21%), but did not differ (P>0.05) significantly from 9 days (76.25±1.46%). However, fertility was lowest (P<0.05) at 12 days insemination interval (67.79±1.76%). There was no significant effect of frequency of insemination on hatch of fertile eggs. Mean values ranged from 79.16±1.83% (3days) to 82.74±1.71% (9days). Hatch of set eggs (%) was higher (p<0.05) at 3days (63.85±2.08%) and 9 days (63.31±1.88%) interval compared to 12 days (55.94±2.39%), which did not differ (p>0.05) significantly from 6 days (61.57±2.26%). Early and Mid - Embryo mortality were not significantly affected by varying insemination frequency and ranged from 5.87±1.10% (9 days) to 8.53±1.23% (3 days) and 7.52%±0.76 (3 day) to 9.39±0.98% (6 days), respectively. However, higher mortality at late embryonic stage was observed (p<0.05) with 3 days insemination interval (4.94±0.81%) compared to 6 days (2.88±0.57%), but was similar ((p>0.05) to 9 days (3.37±0.53%) and 12 days (3.39±0.62%). Chick's quality percentage ranged from 92.02±2.51% (9 days) to 96.54±2.09% (3 days) insemination frequency.

Table 4.6 Table of Inseminated Pooled Semen for Fifteen Weeks

Parameters	Mean Values and Standard Deviation
Sperm concentration (x 10^9 /mL)	0.48 ± 0.17
Sperm Livability (%)	98.6 ± 9.0
Sperm motility (%)	93.1 ± 2.5

Table 4.7 Eggs fertility, Hatch parameters and Embryonic Mortality from Broiler breeders Hens inseminated with 0.02mL Undiluted Semen at Different insemination frequency.

Treatment (Insemination Intervals)	Number of eggs set	Fertility (%)	Hatch of Fertile Eggs (%)	Hatch of Set Eggs (%)	Embryonic mortality (%)			Chicks quality
					Early	Mid	Late	
3 days	1609	80.36±1.8[a]	79.16±1.8	63.85±2.1[a]	8.53±1.1	7.52±0.8	4.94±0.8[a]	96.54±2.1
6 days	1354	74.2 ±1.8[b]	82.36±2.0	61.57±2.1[ab]	6.43±1.3	9.39±0.9	2.88±0.6[b]	94.65±2.1
9 days	1402	76.25±1.5[ab]	82.74±1.7	63.31±1.9[a]	5.87±1.1	8.03±1.2	3.37±0.5[ab]	92.02±2.5
12 days	1550	67.79±2.2[c]	80.91±2.1	55.94±2.4[b]	7.12±1.4	8.87±1.4	3.39±0.6[ab]	90.38±4.0

a, b, c - means in a column with different superscripts are significantly different ($p<0.05$)

4.3 STUDY 3 POTENTIAL OF DEXTROSE AND NORMAL SALINE AS A SEMEN DILUENTS ON QUALITY OF SEMEN, FERTILITY AND HATCH PARMETERS IN BROILER BREEDER CHICKENS

Experiment 1: ***In vitro* Test of Dextrose Saline and 0.9% Saline Use as Semen Diluents**

4.3.1 Spermatozoa Motility at Room -Temperature (27.1^{O}C - 27.8^{O}C)

The Spermatozoa motility of semen diluted with varied diluents and raw semen for a period of time is shown in Table 4.8. At 0 hour and first hour progressive spermatozoa motility did not exhibit any significant effect of the different diluents, with motility ranging from 91.71±1.7% (undiluted semen) to 93.3±1.7% (dextrose saline) and 81.0±2.8% (undiluted semen) to 85.6±1.7% (dextrose saline) respectively. However, at 2 hours, progressive motility was significantly higher ($p<0.05$) in undiluted semen (78.3±1.7%) and dextrose saline (81.7±1.7%) compared to normal saline (74.7±1.7%), but did not differ significantly from modified Ringers solution (76.7±1.7%). Progressive spermatozoa motility observed in undiluted semen, (71.7±1.7%) and normal saline (71.7±1.7%) at 3 hours were similar and higher ($p<0.05$) than other treatments. At the 4th hour, significantly ($p<0.05$) higher progressive spermatozoa motility was observed in undiluted semen (60.0±5.8%) compared to dextrose saline (36.67±3.6%), but did not differ ($p>0.05$) significantly from modified Ringer's solution (51.7±4.4%) and normal saline (53.3±6.0%).

4.3.2 Linear Relationship of Progressive Spermatozoa Motility to Storage Time at Room Temperature (27.1^{O}C to 27.8^{O}C)

The relationship of undiluted semen spermatozoa motility to storage period is shown in Figure 4.4. It was observed that a negative linear relationship existed between spermatozoa motility and storage period, with decrease in motility as the level of storage period increased. The R^2 value (0.95) indicated that a strong association existed between undiluted semen spermatozoa motility and storage period.

The relationship of spermatozoa motility to storage period using modified Ringer's solution as diluent is shown in Figure 4.5. A negative linear relationship was observed as increasing storage period resulted in lowered spermatozoa motility. The R^2 value

(0.98) indicated that a strong association existed between spermatozoa motility and storage period using modified ringer's solution as diluent.

The relationship of spermatozoa motility to storage period using normal saline as diluent is shown in Figure 4.6. A negative linear relationship existed between spermatozoa motility and storage periods. Increasing storage period resulted in lowered sperm motility. The R^2 value (0.92) indicated that a strong association existed between spermatozoa motility and storage period using normal saline as diluent.

The relationship of spermatozoa motility to storage period using dextrose saline as diluent is shown in Figure 4.7. A negative linear relationship existed between spermatozoa motility and storage period. Increasing storage period resulted in lowered spermatozoa motility. The R^2 value (0.97) indicated that a strong association existed between spermatozoa motility and storage period using dextrose saline as diluent.

Table 4.8: Spermatozoa Motility (%) in Stored Raw Semen and Mixed (Diluted) Semen with Different Diluents (Undiluted Semen, Normal and Dextrose Saline and Modified RS) at Room Temperature (27.1^{O}C - 27.8^{O}C)

TREATMENTS	Period				
	Zero hour	one hour	Two hour	Three hour	Four hour
Undiluted semen	91.7 ± 1.7	81.0 ± 2.8	78.3 ± 1.7^{a}	71.7 ± 1.7^{a}	60.0 ± 5.8^{a}
Modified RS	91.7 ± 1.7	81.6 ± 1.7	76.7 ± 1.7^{ab}	61.7 ± 1.7^{b}	51.7 ± 4.4^{ab}
Normal Saline	93.3 ± 1.7	83.3 ± 1.7	74.7 ± 1.7^{b}	71.7 ± 1.7^{a}	53.3 ± 6.0^{ab}
Dextrose saline	91.7 ± 1.7	85.6 ± 1.7	81.7 ± 1.7^{a}	58.3 ± 4.4^{b}	36.67± 3.3^{b}

a,b- mean in a column having different superscripts differ significantly

Means without superscript among the treatment group in a column are not significantly different

Mean ± standard error, Modified RS – Modified ringer solution

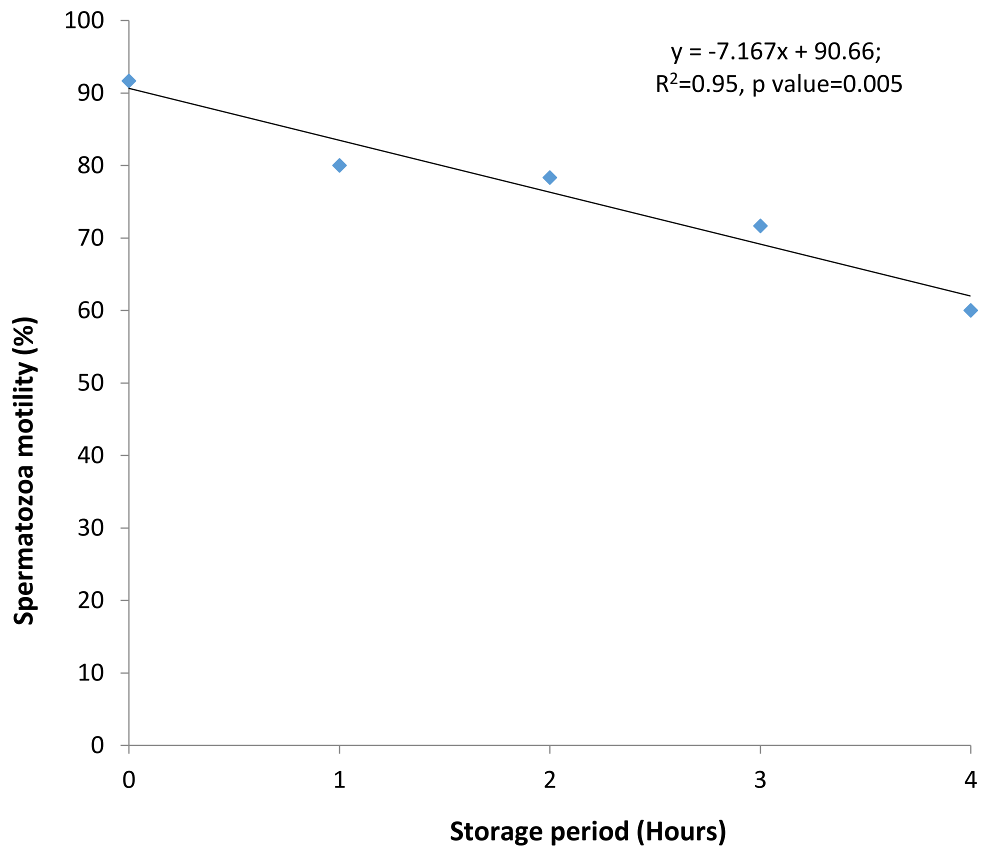

Figure 4.4: Relationship between Spermatozoa Motility and Semen Storage Period in undiluted at Room Temperature (27.1^{O}C to 27.8^{O}C)

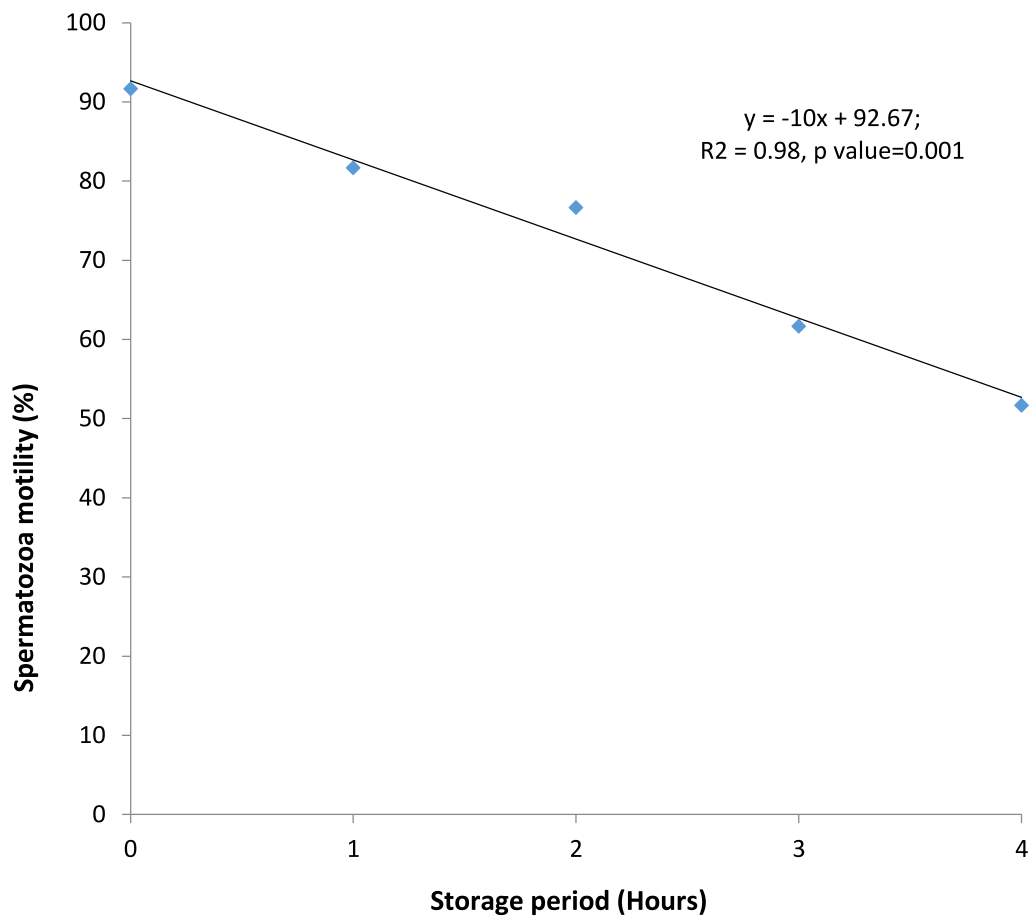

Figure 4.5: Relationship Between Sperm Motility and Storage Period in Semen Diluted with Modified Ringer's Solution at Room Temperature (27.1^{O}C to 27.8^{O}C)

4.3.3 Periodic Evaluation of Sperm Plasma Membrane Integrity (PMI) in Diluted Semen Stored at Room Temperature (27.1^{O}C to 27.8^{O}C) for Four Hours

The periodic assessment of integrity of plasma membrane of sperm as affected by types of diluents is shown in Figure 4.8. It was observed that plasma membrane integrity of spermatozoa in T_1 (98.00%) was higher ($p<0.05$) than T_3 (95.00%), but did not differ significantly from T_2 (97.00%) and T_4 (95.60%) at 0 hour. However, at I hour diluents did not affect sperm plasma membrane integrity and the value ranged from 93.5% (T_3) to 97.00% (T_1).

At 2 hours, sperm plasma membrane integrity (IPM) in T_1 (97.5%) was higher ($p<0.50$) compared to T_3 (93.00%) and T_4 (93.70%), but was not significantly different from T_2 (96.00%). At 3 hours, similar sperm PMI was observed in T_1 (95.60%) and T_2 (95.10%) compared to T_3 (91.70%). However, at 4 hours, there was no significant effect of diluents on plasma membrane integrity of sperm cells across different treatments.

4.3.4 Liveability of Sperm from undiluted Semen and Semen Diluted with Different Solutions at Room Temperature (27.1^{O}C to 27.8^{O}C) and Held Four Hours

The periodic evaluation of sperm liveability as affected by different types of diluents is shown in Figure 4.9. It was observed that sperm liveability was not influenced by diluent types at different storage intervals values ranges from (95 - 100%).

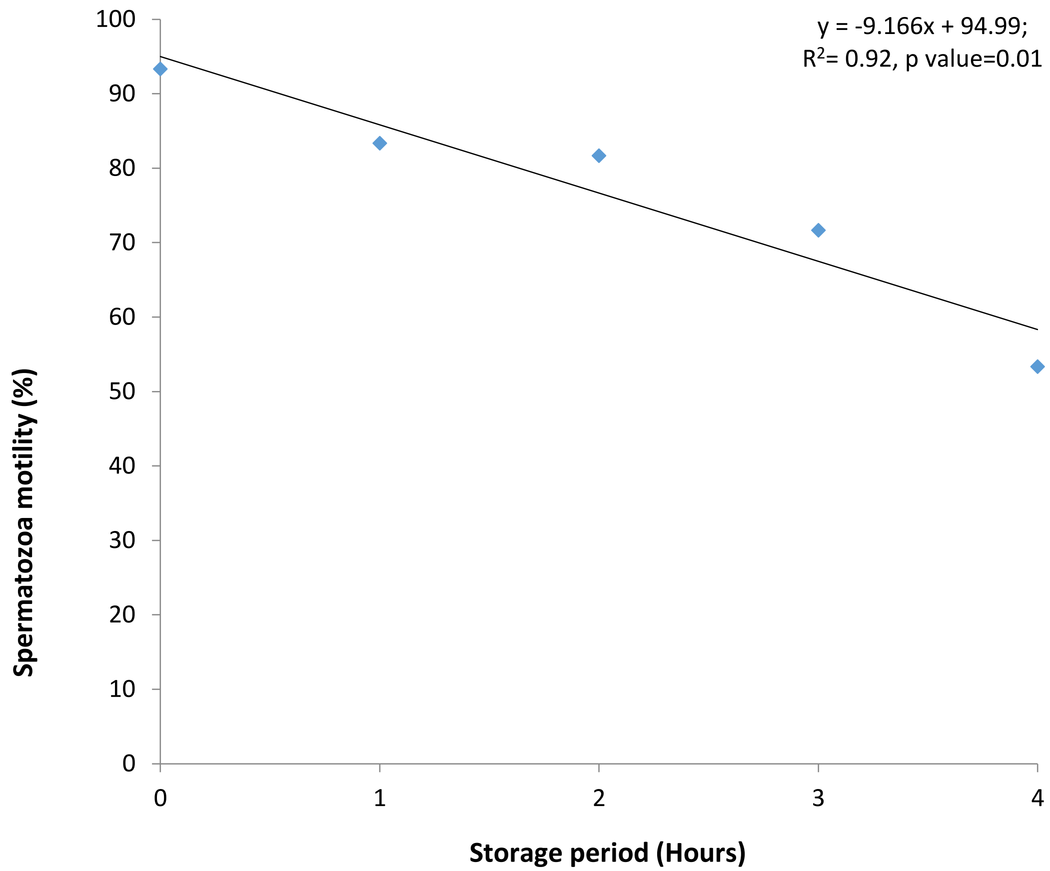

Figure 4.6: Relationship Between Spermatozoa Motility and Storage Period of normal Saline Diluted Semen at Room Temperature (27.1^{O}C - 27.8^{O}C)

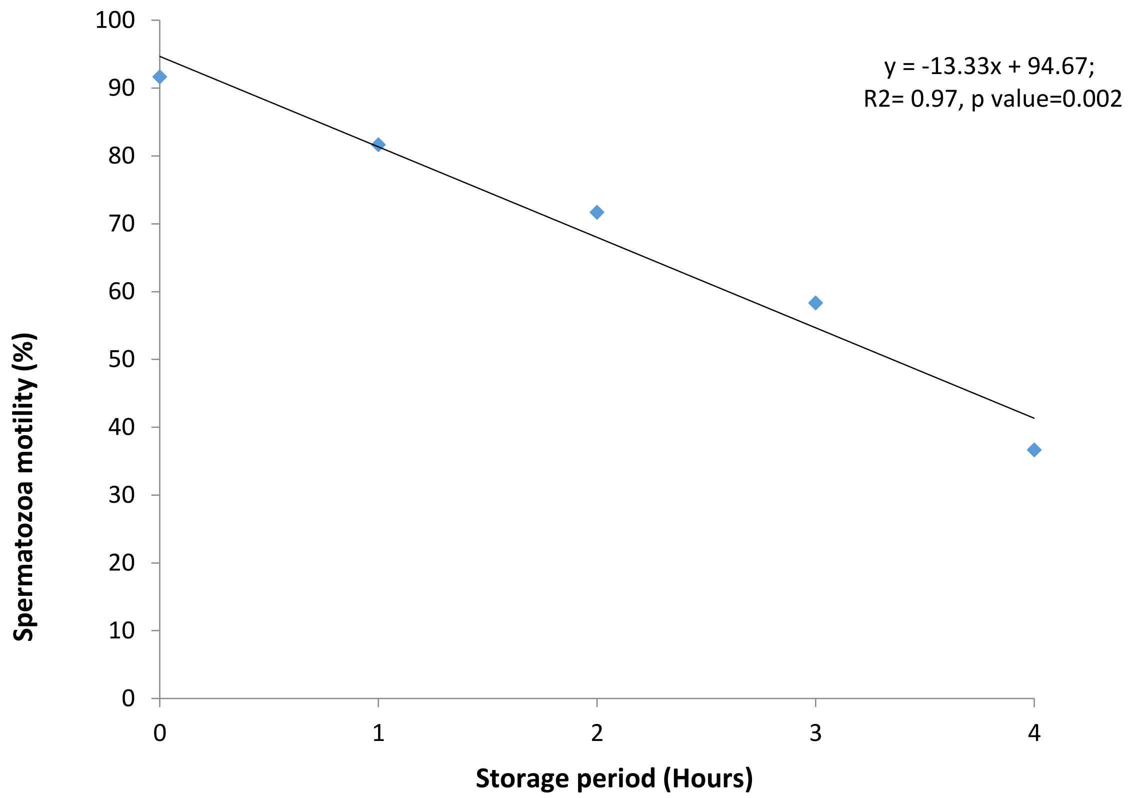

Figure 4.7: Relationship between Spermatozoa Motility and Storage Period of Dextrose Saline Diluted Semen at Room Temperature (27.1^{O}C - 27.8^{O}C)

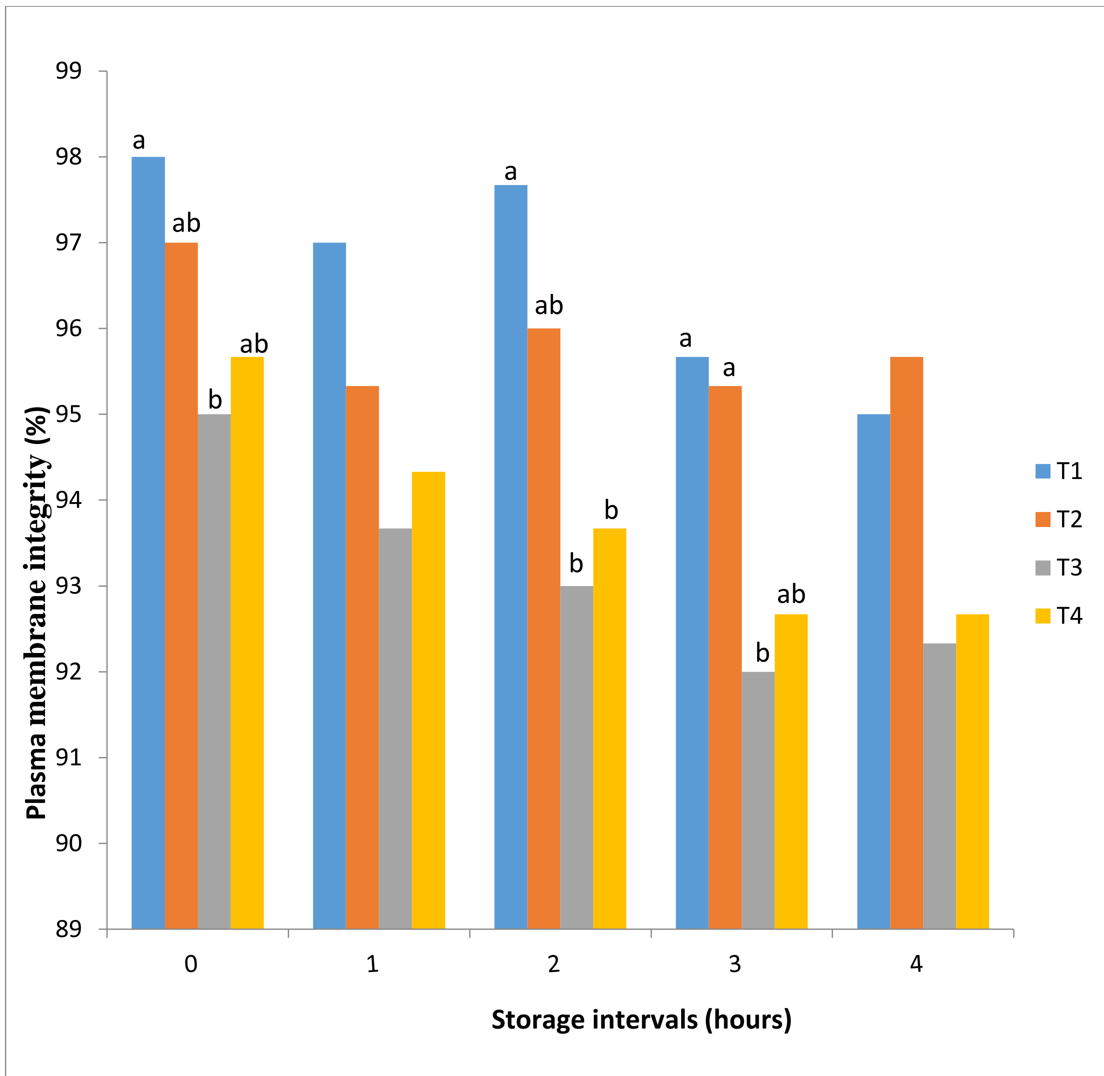

Figure 4.8: Periodic Assessment of Sperm Plasma Membrane Integrity as Influenced by Diluents and Storage Time, at Room Temperature (27.1^{O}C - 27.8^{O}C)

T1-Undiluted Semen (1:0), T2- Ringer's Solution (Modified) (1:1), T3 – 0.9% Saline (1:1), T4 – Dextrose Saline (1:1)

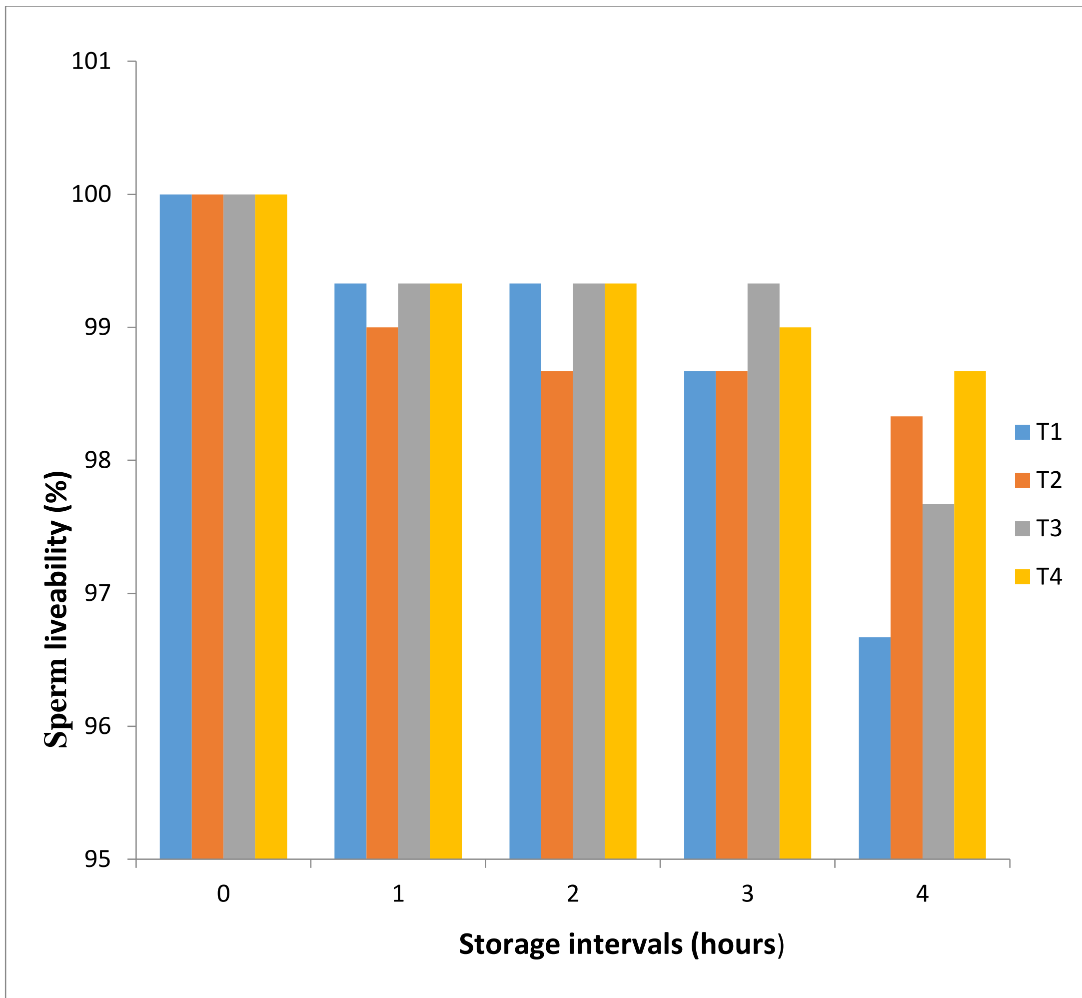

Figure 4.9: Periodic Evaluation of Sperm Liveability as Affected by Different Types of diluents

T1-Undiluted Semen (1:0), T2- Ringer's Solution (Modified) (1:1), T3 – 0.9% Saline (1:1), T4 – Dextrose Saline (1:1)

Experiment 2: Influence of Normal Saline (0.9% Nacl) and Dextrose Saline as Semen Diluents on Fertility Parameters in Broiler Breeder Hens

4.3.5 Fertility (%) of Eggs from Broiler Breeder Hens inseminated with Dilutedand Undiluted Semen at 1:1 for two Successive day with Different Diluents

The effect of different semen diluents at 1:1 dilution ratio on percentage fertility of broiler breeder eggs is shown in Table 4.9. Different semen diluents did not significantly ($p>0.05$) affect egg fertility up till 7 days post-insemination, the value ranging from 70.95±6.67% (T_1) to 85.4±7.62% (T_4). At the 2nd week post-insemination, egg fertility revealed no significant effect of the semen diluents on fertility with value that ranged from 28.21±1.69% (T_2) to 37.38±5.35% (T_3). However, at the 3rd week, ($p<0.05$) egg fertility was higher ($p<0.05$) in T_4 (4.49±1.55%) compared to T_1 (0.00±0.00%) and T_3 (0.00±0.00%), but not significantly different from T_2 (2.08±2.08%) and T_5 (1.14±1.14%). There was no observable fertility at the 4th week post-insemination.

4.3.6 Polynomial Regression of Fertility on Post Insemination Period in BroilerBreeder Hens Inseminated with Undiluted Semen 0.01mL

The relationship between days of egg collection post - insemination and fertility in broiler breeder hens is shown in Figure 4.10. Highest fertility (58%) was observed at 5 days post-insemination. The R^2 value (0.58) indicated that about 58% of the determinants of fertility was attributed to the effect of days after insemination.

4.3.7 Linear Regression of Fertility on Period of Egg Collection Post - Insemination in Broiler Breeder Hens Inseminated with Undiluted Semen

The relationship between fertility and days after insemination with undiluted semen is shown in Figure 4.11. A linear relationship between fertility and days after insemination was observed with undiluted semen. The R^2 value (0.74) indicated that a strong association existed between egg fertility and post-insemination period.

The relationship of fertility to days of egg collection post insemination with normal saline dilution at 1:1 is shown in Figure 4.12. A linear relationship was observed between fertility and eggs collection after insemination. Increasing post-insemination

egg collection days resulted in decline in fertility. The R^2 value (0.79) indicated that a strong association existed between fertility and post-insemination days.

The relationship of fertility to days of egg collection after insemination using dextrose saline diluent at ratio 1:1 is shown in Figure 4.13. It was observed that a negative linear relationship existed between fertility and days after insemination. The R^2 value (0.77) indicated that a strong association existed between fertility and egg collection period after insemination with dextrose saline (1:1).

The relationship of fertility to days after insemination using modified ringer's solution as diluent (1:1) is shown in Figure 4.14. A linear relationship existed between fertility and days of eggs collection after insemination, using modified ringer's solution as diluent (1:1). Fertility decreased with increasing number of egg collection days post-insemination. The R^2 value (0.65) indicated that about 65% of the determinants of fertility was attributed to post-insemination days of egg collection.

Table 4.9: Percentage Fertility of Eggs from Broiler breeder Hens Post Insemination with Diluted (1:1 with various diluents) and Undiluted Semen after Two Consecutive Days

	Treatments				
Weeks after artificial Insemination	Undiluted (0.01mL)	Undiluted (0.02mL)	0.9% saline (0.02mL)	1%Dextrose saline (0.02mL)	Modified Ringer's solution (0.02mL)
1	70.95 ± 6.67	75.18 ± 9.50	76.18 ± 8.15	85.62 ± 7.03	80.74 ± 7.62
2	35.42 ± 5.24	28.21 ±1.69	37.38 ± 5.35	36.05 ± 4.22	28.83 ± 6.07
3	0.00 ± 0.00^{b}	2.08 ± 2.08^{ab}	0.00 ± 0.00^{b}	4.49 ± 1.55^{a}	1.14 ± 1.14^{ab}
4	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

a, b- Means in a row with different superscripts are significantly different ($p < 0.05$)

(Means±SE) - Standard Error

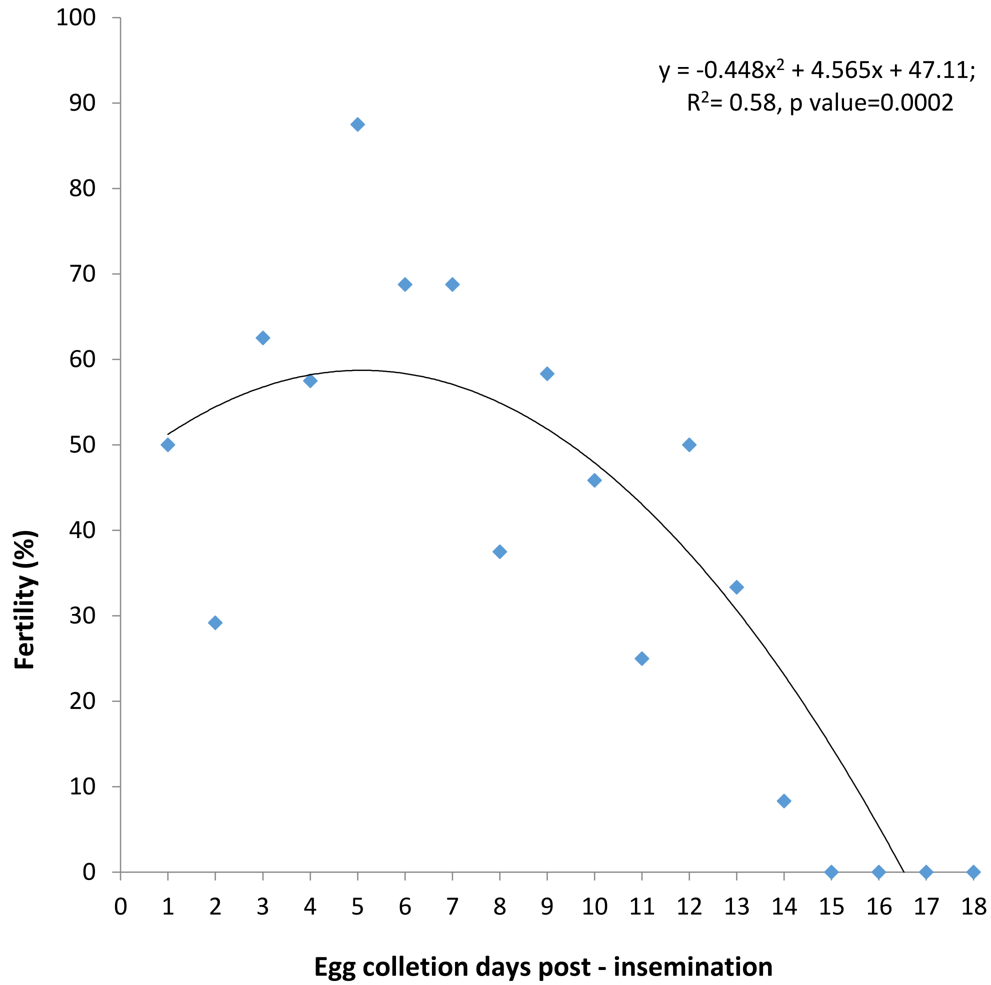

Figure 4.10: Relationship of Fertility to Egg Collection Days Post - Insemination in Broiler Breeder Hens Inseminated with 0.01mL of Undiluted Semen

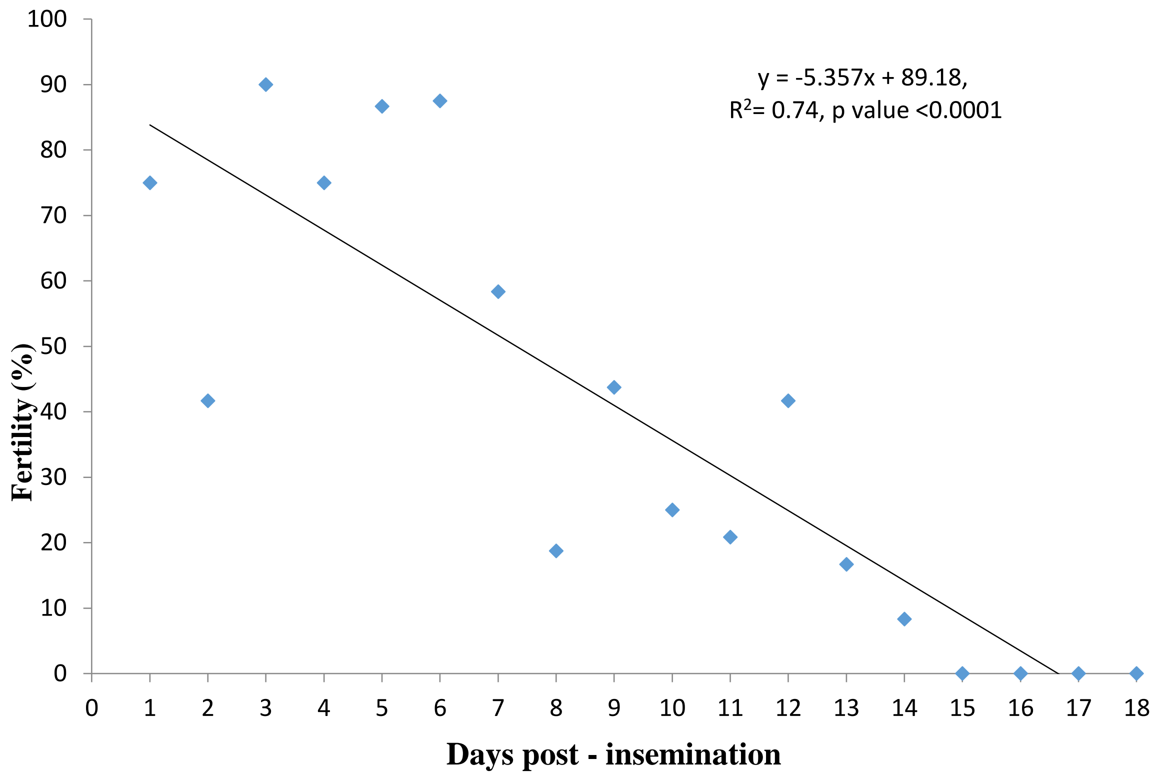

Figure 4.11: Relationship of Fertility to Egg Collection Days Post – insemination in Broiler Breeder Hens Inseminated with 0.02mL of Undiluted Semen

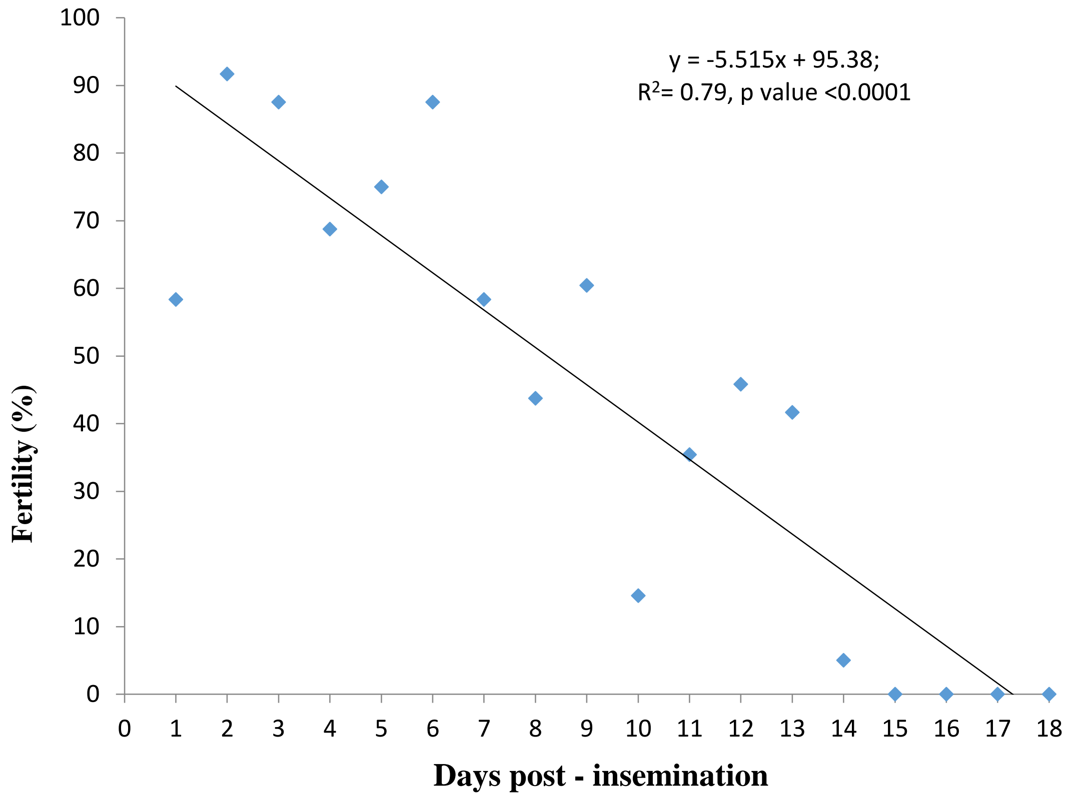

Figure 4.12: Relationship of fertility to days post - insemination of broiler breeder hens inseminated with 0.02mL of Normal saline Diluted semen in ratio 1:1

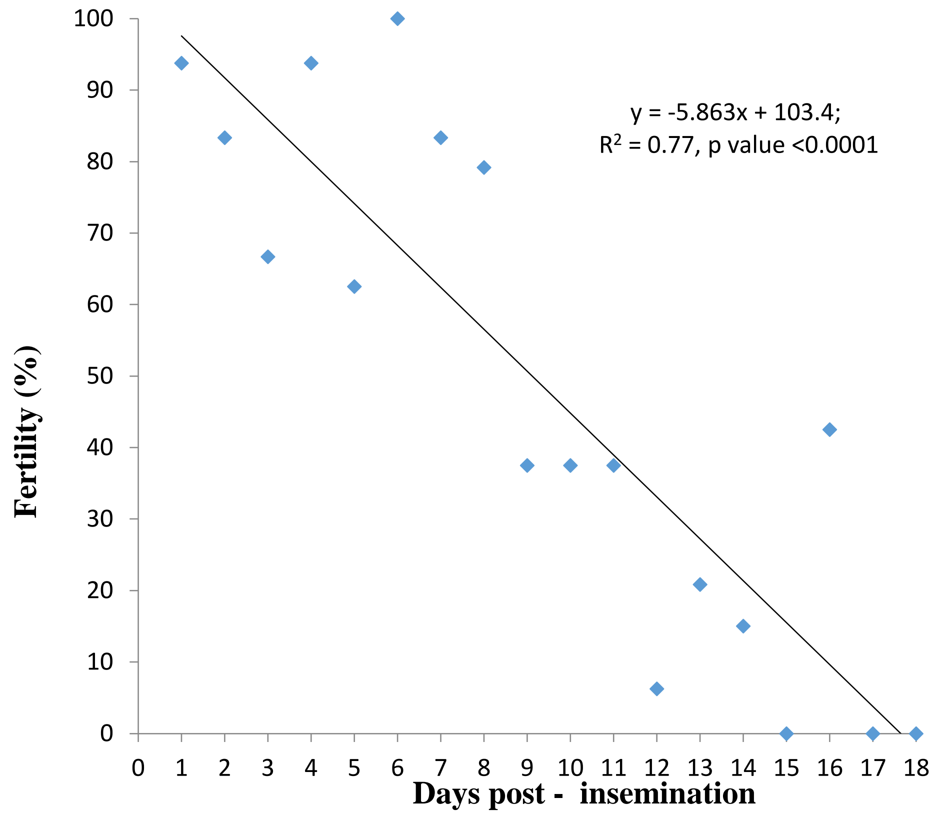

Figure 4.13: Relationship of Fertility to Egg Collection Days Post – Insemination in Broiler Breeder Hens Inseminated with 0.02mL of Dextrose Saline Diluted Semen at 1:1

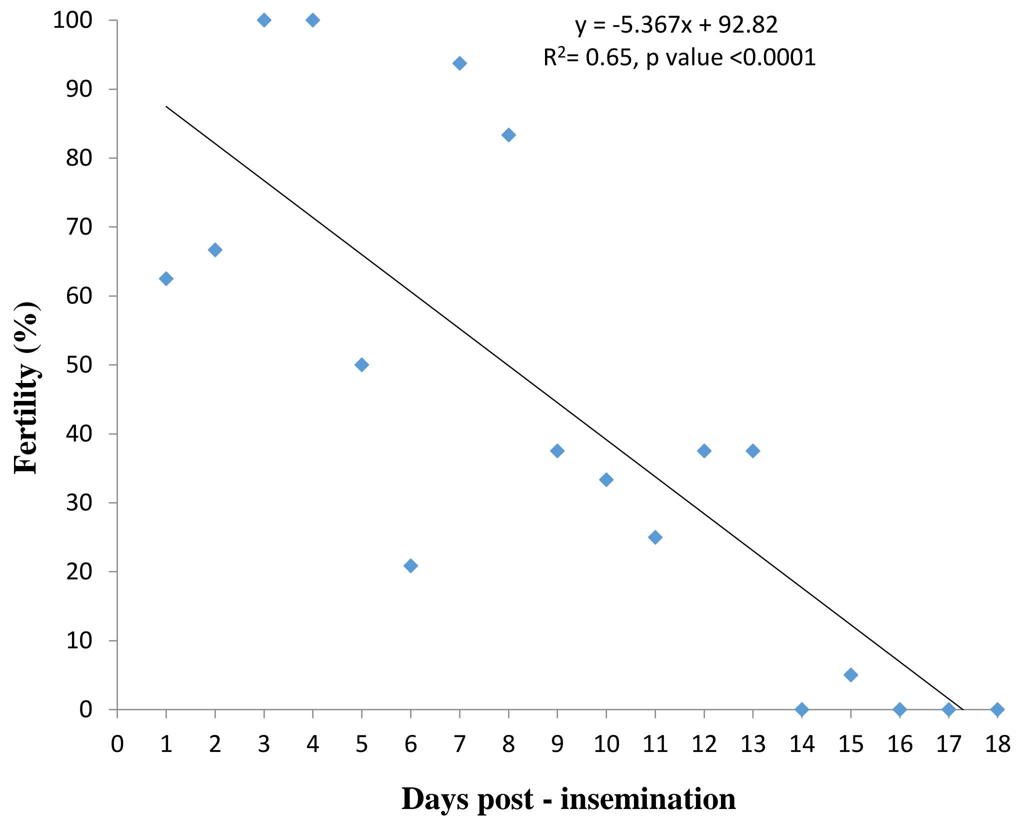

Figure 4.14: Relationship of Fertility to Egg Collection Day's Post - Insemination of Broiler Breeder Hens Inseminated with 0.02mL of Modified Ringer's Solution Diluted Semen at 1:1

4.3.8 Hatchability of Eggs From Broiler Breeder Hens Inseminated with Undiluted and Diluted Semen (1:1).

Values of hatchability are presented in Table 4.10. At the first 7 days, there was no significant ($p>0.05$) differences observed for hatchability of eggs from different semen treatments and values ranged from 83.42±5.82% (T_3) to 96.60±2.06% (T_4).

At the 2nd and 3rd week, different semen diluents had no significant effect on hatchability of eggs, while there were no fertile eggs collected by the 4th week post – insemination which returned zero hatchability.

Table 4.10: Hatchability of Eggs from Broiler Breeder Hens Inseminated with Undiluted and Diluted semen 1:1 with Varying Diluents

Weeks Post - insemination	Treatments				
	Undiluted semen (0.01mL)	Undiluted semen (0.02mL)	0.9% saline (0.02mL)	1% Dextrose saline (0.02mL)	Modified Ringer's solution (0.02mL)
1	87.7 ± 7.278	91.13 ± 3.29	83.42 ± 5.82	96.60 ± 2.06	93.00 ± 2.60
2	95.00 ± 5.00	100.00 ± 0.00	92.92 ± 4.73	86.81 ± 5.24	71.43 ± 24.05
3	0.00 ± 0.00	25.00 ± 25.00	0.00 ± 0.00	50.00 ± 28.87	50.00 ± 28.87
4	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

(a, b) – Means in a column with different superscripts differs (P<0.05

(Mean ± SE)

STUDY 4: FERTILITY RESPONSE OF BROILER BREEDER HENS TO`SEMEN DILUTION RATIO AND INSEMINATION FREQUENCY

4.4.1 Effect of Varied Dilution Ratio of Broiler Breeder Semen on Hourly Progressive Spermatozoa Motility at Room Temperature (27.4^{O}C – 28.0^{O}C)

The periodic evaluation of progressive spermatozoa motility of broiler breeder semen extended at different dilution ratios with 1% dextrose saline is shown in Figure 4.15. At 0 hour, progressive sperm motility was significantly ($p<0.05$) higher in $T_{1\ (1:0)}$ (90.0%), $T_{2\ (1:1)}$ (87.0%) and $T_{3\ (1:2)}$ (83.0%) compared to $T_{4\ (1:3)}$ (70.0%). At the first hour, spermatozoa motility observed in T_1 (84.0%) was significantly ($p<0.05$) higher compared to other treatments. The lowest ($p<0.05$) spermatozoa motility was observed in T_3 (55.0%) and T_4 (52.0%). At 2 hours of storage, the T_1 (58.0%) had significantly ($p<0.05$) greater progressive spermatozoa motility compared to other treatments. However, there were no significant ($p>0.05$) differences in spermatozoa motility observed in T_2 (52.0%), T_3 (47.0%) and T_4 (48.0%) at 2 hours. At the third hour, increased spermatozoa motility was observed ($P<0.05$) in T_1 (55.0%) compared with other treatments while at the 4th hour, the T_1 (42.0%) and T_2 (41.0%) had similar values for spermatozoa motility and both were higher ($P<0.05$) than T3 (37.0%) and T_4 (32.0%).

4.4.2 Characteristics of Pooled Semen Prior To Dilution and Insemination for Ten Weeks

The characteristics of pooled semen used for dilution and insemination for ten weeks are shown in Table 4.11. The mean value of sperm concentration observed was 0.76 ($x10^9$/ml), while sperm cell liveability value was 94.3±7.8%. The mean value of sperm motility was 90.00±2.7%

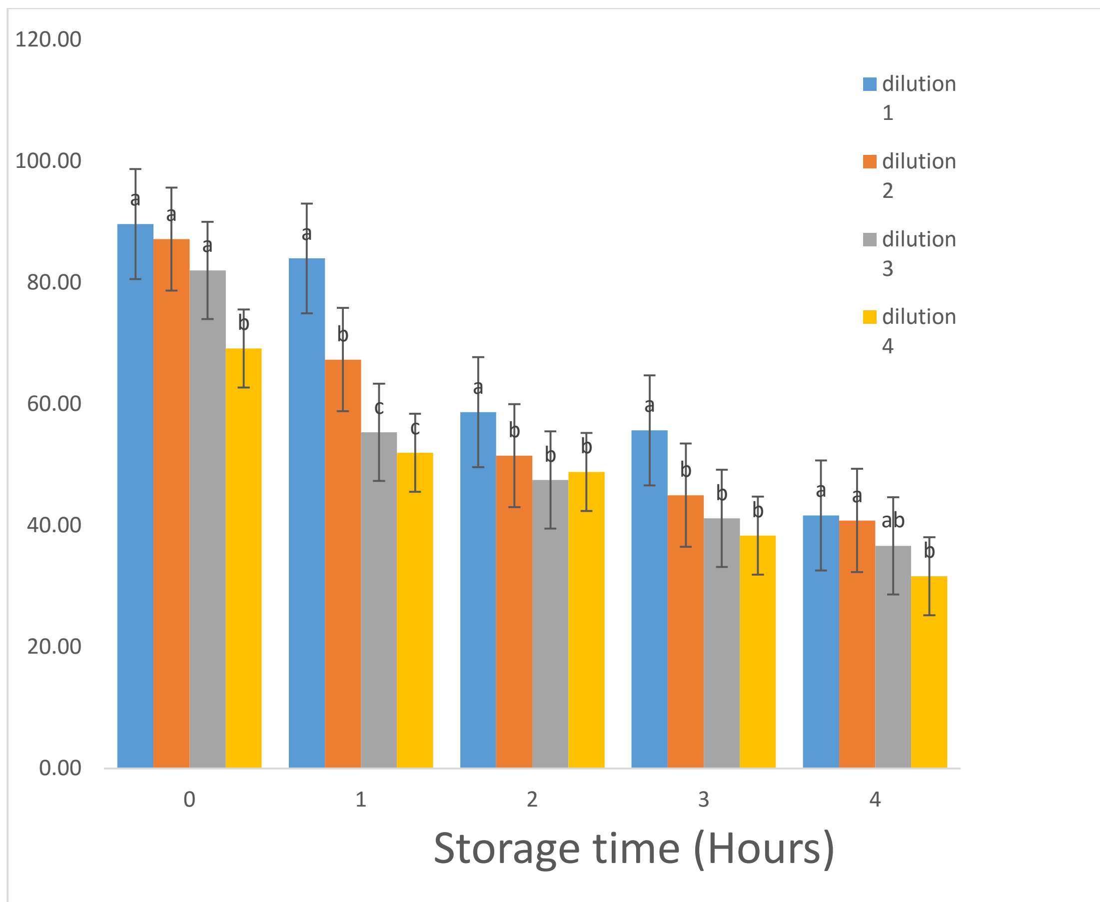

Figure 4.15: Periodic Evaluation of Spermatozoa Motility of Broiler Breeder Semen Extended with 1% Dextrose Saline at Different Dilution Ratios

[a,b,c] Means across the bars at each storage time with different alphabets are significantly different ($p<0.05$)

Dilution 1- Uudiluted Semen (1:0), Dilution 2 – semen and dextrose saline (1:1),

Dilution 3 - semen and dextrose saline (1:2) and Dilution 4 – semen and dextrose saline (1:3)

Table 4.11: Characteristics of Pooled Semen Used for Dilution and Insemination over Ten Weeks

Semen parameters	Mean Values (± Standard Deviation)
Sperm concentration (x 10^9 cells/mL)	0.76±2.4
Livability (%)	94.3±7.8
Sperm motility (%)	90.0±2.7

4.4.3 Fertility and Hatch Parameters as Influenced by Semen Dilution Ratio and Insemination Frequency

Main effects of dilution ratios and frequency of insemination on fertility in broiler breeder hens are shown in Table 4.12. Percentage fertility was not significantly (p>0.05) affected by varying dilution ratios and values ranged from 62.94±5.74% (1:1) to 69.17±0.58% (1:2). However, different insemination intervals significantly (p<0.05) affected fertility of eggs. Higher fertility was observed (P<0.05) in three days (75.6 ± 4.27%) insemination frequency compared to 6 days (60.59±4.39%), but did not differ (p>0.05) significantly from 9 days (64.15±2.79%). Hatch of fertile eggs (%) observed was not influence by insemination intervals. The values ranged from 6 days (87.16±4.46%) to 9 days (88.30 ±1.73%) insemination interval. Semen dilution ratio effect on hatch of fertile eggs (%) ranged from 85.83±4.43% (1:1) to 87.66±1.78% (1:0). The various dilution ratio had no significant effect on hatch of set eggs, However insemination intervals of 3 days (69.87±4.52%) which was significantly(p<0.05) higher than 6 days (56.09±3.96) but was similar to 9 days (59.31±2.84%) intervals. The interaction effect of insemination frequency and dilution ratio on eggs fertility in broiler breeder hens is presented in Table 4.12. Showed higher fertility (%) at (P<0.05) in 3days at ratio 1:1 (75.78±8.23%) and 3days at ratio 1:0 (70.63±9.49%) compared to 6 days at ratio1:1 (50.65±10.84%). Hatch of fertile (%) was not significantly impacted by the interaction of insemination interval and dilution ratio, and values ranged from 80.77±13.58% at 6 days (1:1) to 93.22±1.35% at 6 days (1:2). Percentage hatchability of set eggs observed in 3 days (1:2) (73.07±6.22%) was significantly (p<0.05) higher than 6 days (1:1) (47.97±9.85%), but was similar to other combinations.

Table 4:12: Semen Dilution Ratio and Insemination Frequency: It's Interaction Effect on Egg Fertility and Hachability in Broiler Breeder Hens

Factors			Parameters		
			Fertility (%)	Hatch of fertile (%)	Hatch of set (%)
Insemination Interval	3days		75.69±4.27^{a}	87.30±2.22	69.87±4.52^{a}
	6days		60.59±4.39^{b}	87.16±4.46	56.09±3.96^{b}
	9days		64.15±2.79^{ab}	88.30±1.73	59.31±12.84^{ab}
Semen Dilution ratio	1:0		68.32±3.08	87.66±1.78	62.72±3.71
	1:1		62.94±5.73	85.83±4.43	59.01±5.46
	1:2		69.17±0.58	89.28±2.10	63.54±3.48
Insemination Intervals x Semen Dilution ratio	3	1:0	70.63±9.49^{ab}	91.78±2.73	65.19±10.89^{ab}
		1:1	75.78±8.23^{a}	86.82±4.75	71.34±8.42^{ab}
		1:2	62.39±7.15^{ab}	83.31±3.32	73.07±6.22^{a}
	6	1:0	64.25±0.37^{ab}	87.49±3.73	58.54±3.10^{ab}
		1:1	50.65±10.84^{b}	80.77±13.58	47.97±9.85^{b}
		1:2	66.86±6.12^{ab}	93.22±1.35	61.76±5.45^{ab}
	9	1:0	70.09±3.28^{ab}	83.71±0.65	64.41±4.86^{ab}
		1:1	62.39±7.15^{ab}	89.89±2.59	57.72±7.25^{ab}
		1:2	59.95±1.78^{ab}	91.31±3.55	55.79±0.32^{ab}

a, b – mean in a column with different superscripts are significantly different ($p<0.05$)

4.4.4 Dilution Ratio of Semen and Frequency of Insemination: It's Effects on Embryonic Mortality in Broiler Breeders

Main effects of semen dilution ratio and frequency of insemination and their interaction on embryo death is shown in Table 4.13. These had no significant (P>0.05) effect on early, mid, late or total embryonic mortality. Values recorded for total embryonic mortality across treatment groups ranged from 5.53% (1:1) to 8.63% (1:0)

Interaction between semen dilution ratio and frequency of insemination on embryonic mortality is shown in Table 4.14. It was observed that early, mid, and late embryonic mortality in total were all not significantly affected by dilution ratio and insemination interval interaction. The values ranged from 0.62 to 2.33% (Early), 3.79 to 7.03% (Mid), 0.00 to 1.43% (Late) and 4.41 to 9.29% (Total), respectively.

Table 4.13: Main Effects of Dilution Ratio of Semen and Frequency of Insemination on Embryonic Mortality of Broiler Breeder Hens

		Embryonic mortality (%)			
Insemination frequency		Early	Mid	Late	Total
	3 days	1.68±0.66	5.52±1.81	0.56±0.27	7.76±2.31
	6days	1.65±0.71	4.72±1.35	0.29±0.19	6.66±1.66
	9 days	1.48±0.45	5.84±1.47	0.68±0.49	7.99±1.69
P value		0.97	0.89	0.78	0.89
Semen dilution ratio					
	1:0	1.98±0.59	6.25±1.63	0.40±0.27	8.63±2.00
	1:1	1.09±0.39	3.92±0.94	0.51±0.35	5.53±0.97
	1:2	1.73±0.76	5.91±1.85	0.61±0.48	8.25±2.32
SEM		1.14	2.99	0.68	3.64
P-value		0.63	0.59	0.93	0.54

Table 4.14: Interaction Effect of Frequency of Insemination and Dilution Ratio on Embryonic Death in Broiler Breeder Hens

Frequency of insemination	Dilution ratio	Embryonic death (%) Early	Mid	Late	Total
3days	1:0	1.87±0.86	5.61±2.12	0.74±0.74	8.22±2.73
	1:1	0.93±0.93	3.92±2.09	0.93±0.93	5.78±2.14
	1:2	2.25±1.77	7.03±5.29	0.00±0.00	9.29±6.97
6days	1:0	2.02±1.49	6.32±3.89	0.46±0.46	8.79±4.85
	1:1	0.62±0.62	3.79±2.22	0.00±0.00	4.41±1.72
	1:2	2.33±1.60	4.04±0.02	0.41±0.41	6.78±1.35
9days	1:0	2.07±1.09	6.82±3.49	0.00±0.00	8.88±4.11
	1:1	1.74±0.55	4.05±1.09	0.62±0.62	6.41±1.65
	1:2	0.62±0.62	6.65±3.21	1.43±1.43	8.69±3.55
SEM		1.14	2.99	0.68	3.67
P-value		0.93	0.98	0.84	0.98

4.4.5 Concentration of Spermatozoa ($X10^6$ Cell/ML) In Recovered Fluid from Different Segment of the Oviduct of Female Broiler Breeders Inseminated with Diluted Semen at Different Insemination Frequency.

Main effects of semen dilution and insemination frequency on oviductal sperm count in breeding broiler hens are presented in Table 4.15. Infundibulum sperm concentration was not affected by dilution ratios and insemination intervals (P>0.05) and results ranged from 0.69±0.08 to 0.87±0.08 and 0.67±0.07 to 0.83±0.08 respectively. Sperm concentration in magnum /Isthmus segment was not significantly affected by varying dilution ratios, and ranged from 0.84±0.07 (1:1) to 1.07±0.10 (1:2). However, insemination intervals significantly (p<0.05) affected concentration. However, Sperm count was significantly(P < 0.05) higher in the magnum /isthmus segment of breeder hens inseminated at 3 days (1.07±0.11) and 6 days frequency (1.16± 0.09), compared to 9 days (0.66±0.04). Sperm concentration observed in the uterus/vagina was not influenced by varied dilution ratios, the mean values were 2.28±0.29 (1:2) to 2.84±0.23 (1:0). However, the uterus/vagina spermatozoa count was significantly (P<0.05) higher with 9days insemination frequency (3.31±0.29) compared to 6 days (1.84±0.16) and 3 days (2.62±0.21).

The interaction of semen dilution ratio and insemination frequency had significant effect on the oviductal sperm concentration in all the segments of the oviduct in broiler breeder hens. Sperm concentration observed in the infundibulum of hens ranged from 0.36 to 1.19, in the magnum /isthmus was 0.50 to 1.29 at the uterus/vagina segment, values ranged from 0.95 to 4.53.

Uterus/vagina sperm concentration was significantly (p<0.05) affected by dilution ratio and insemination interval interaction. Sperm concentration observed in uterus/vagina of broiler breeder hens on T_9 (4.53) differ significantly (p<0.05) from other treatments, with sperm concentration observed at 1:2 at 3 days (0.96±0.12) and 1:2 at 6 days (0.95±0.11). The main effects of frequency of insemination and semen dilution ratio on sperm concentration in the (UVJ) uterovaginal segment of broiler breeder hens. Higher (p<0.05) sperm count was observed in uterovaginal segment of hens on 1:1 (54.19±5.26) and 1:2 (58.57±11.73) dilution ratio compared to 1:0 (33.86±2.38). However, insemination intervals did not significantly (p>0.05) affect sperm concentration in the uterovaginal segment and values ranged from 44.14±12.07 (9 days) to 58.59±5.19 (3 days). There are interaction outcomes of the dilution-ratio

and insemination interval of the sperm count in uterovaginal segment of broiler breeder hens. Ratio 1:1 at 3 days (109.17±8.73) resulted in higher sperm concentration in the uterovaginal segment when compared to other treatments (P<0.05). However, the lowest (p<0.05) sperm count was observed in 1:0 at 9days (18.92±3.95) and 1:1 at 9days (22.90±2.49) which did not differ (p>0.05) significantly from 1:0 at 3days (39.57±4.01), 1:1 at 6 days (31.59±3.92) and 1:2 at 3 days (26.57±5.19).

Table 4.15: Concentration of Spermatozoa (X10^{6} Cell/ ML) In Recovered Fluids from Different Segment of the Oviduct and Sperm Storage Tubules (SST) of Female Broiler Breeder Inseminated with Semen of Varied Dilution Ratios and Frequency of Insemination.

Factors	Level		Oviductal segments			Uterovaginal Junction SST
			Infundibulum	Magnum/Isthmus	Uterus/Vagina	
Insemination Frequency	3 days		0.77±0.07	1.07±0.11^{a}	2.62±0.21^{b}	58.59±5.19
	6 days		0.67±0.07	1.16±0.09^{a}	1.84±0.16^{c}	44.29±2.70
	9 days		0.83±0.08	0.66±0.04^{b}	3.31±0.29^{a}	44.14±12.07
P value			0.53	<0.0001	<0.0001	0.23
Semen dilution ratio	1:0		0.72±0.08	0.91±0.07	2.84±0.23	33.86±2.38^{b}
	1:1		0.87±0.08	0.84±0.07	2.71±0.17	54.19±5.26^{a}
	1:2		0.69±0.08	1.07±0.10	2.28±0.29	58.57±11.73^{a}
P value			0.09	0.06	0.06	0.03
Insemination Intervals x Semen Dilution Ratio	3	1:0	0.62±0.03^{cd}	0.78±0.17^{b}	2.47±0.31^{c}	39.57±4.01^{cd}
		1:1	0.99±0.17^{ab}	1.19±0.14^{a}	3.84±0.29^{ab}	109.17±8.73^{a}
		1:2	0.65±0.14^{c}	1.23±0.27^{a}	0.96±0.12^{d}	26.57±4.02^{cd}
	6	1:0	0.36±0.04^{d}	1.24±0.12^{a}	3.09±0.23^{bc}	41.10±3.23^{c}
		1:1	1.19±0.12^{a}	0.74±0.09^{b}	1.37±0.22^{d}	31.59±3.92^{cd}
		1:2	0.62±0.15^{c}	1.29±0.16^{a}	0.95±0.11^{d}	59.77±5.19^{b}
	9	1:0	1.17±0.20^{a}	0.72±0.05^{b}	2.97±0.57^{bc}	18.92±3.95^{d}
		1:1	0.52±0.05^{cd}	0.50±0.03^{b}	2.46±0.16^{c}	22.90±2.49^{d}
		1:2	0.81±0.11^{b}	0.73±0.10^{b}	4.53±0.59^{a}	87.23±33.29^{ab}
SEM			0.13	0.14	0.36	12.31
P value			<0.0001	<0.0001	<0.0001	<0.0001

$^{a-d}$ - Means of treatments in columns with different superscripts differed significantly (P<0.05)

CHAPTER FIVE

DISCUSSION

5.1: Effect of Dose of Semen Inseminated on Fertility of Broiler Breeder Eggs from Hens Inseminated for Two Consecutive Days

Semen quality parameters such as sperm concentration, liveability and morphology are good predictors of the fertilizing ability of sperm cells (Liu *et al.,* 2008). The characteristics of pooled semen inseminated on two successive days revealed that the semen ejaculated by the cocks were of good quality and suitable for insemination. The mean values of sperm concentration observed was 0.28±0.13 ($x10^9$ /ml), while sperm cell liveability (%) and motility values were 96.8±3.7% and 90.0±0.0%, respectively. The semen evaluation indicates that 0.02mL, 0.04mL, 0.06mL and 0.08mL of fresh semen contained approximately, 5.6 $x10^6$, 11.2 $x10^6$, 16.8 $x10^6$ and 22.4 $x10^6$ sperm cell, respectively. The non significant variance in fertility across the treatments up to week four post- insemination observed in this study contradicted the report of Saleh *et al.* (2012) and Tabatabaei (2010) who reported that the maximum fertility of eggs was attained with the use of 50 and 100 $\times10^6$ sperm cells. This is also contrary to the report by Bakst *et al.* (2010) that the duration of fertility is directly linked to sperm storage-tubules (SST) number in their corresponding utero-vaginal joint folds. Though, this result was in agreement with the observation of Ewuola *et al*. (2020) that there was no significant difference in fertility with undiluted semen doses ranging from 0.02 mL to 0.08mL in Nera Black breeder chickens.

Brillard and Bakst (1990) reported that the amount of sperm that enter the sperm storage tubules determine the length of the period of fertility in chickens. Initially, it was taught that multiplication of semen doses may possibly make more sperm cells available in the storage tubules. This suggests that increasing the number of spermatozoa for insemination in chickens may sustain a high level of fertility for an extended period. This was different from the result obtained from this current work. Increasing the administered dose of semen across the treatment groups did not improve fertility among the groups.

This suggests that insemination dose in excess of 0.02mL (approximately 5.6 x10^6 motile sperm cell) properly exceeded of the upper limit of spermatozoa number that the SST can accommodate at any given period. Also, by inseminating 0.02ml, more hens will be inseminated compared with higher doses since the fertility is similar, semen wastage will be minimised and ultimately, cost of production for farmers will be reduced. The relative reduction in fertility obtained by the natural mating in this study could be as a result of insufficient sperm cells in the ejaculate, during the two consecutive matings, to fill the SST. This reduction in volume could have been that the actual volume of semen ejaculated naturally was low which supported the basis for this work. During natural mating, cocks are allowed to chase after the hen and incomplete mating are likely to occur and also, stress might be attributed to low semen volume. Inspite of this however, duration of fertile period did not differ compared with the birds inseminated. This is an indication that oviductal sperm age and not SST sperm count determines the maximum duration of fertility. This correspond with the result of Bramwell (2002) where zero fertility was recorded at day 22 post insemination.

5.2: Fertile Period and Effect of Spermatozoa Age in Oviduct on Fertility and Hatchability of Set in Broiler Breeder Hens

In the present study, there was an inverse relationship between oviductal sperm age and fertility up to 22days. Optimum fertility was obtained between 1-7days without repeated insemination which is assumed to be efficient duration of fertile period for Marshall broiler breeder hens. This ascertained that hens artificially inseminated demonstrated effective duration of fertile eggs at days 1-7, respectively. Fertility ranged from 86.98 to 74.1% on days 8 to 9 which slightly decreased to 69.2% on day 10. There was a sharp drop to 5.83% on day 15 and then several fluctuation till fertility reached zero on day 22 which is the duration of fertile period. This implies that there is probably a depletion in the number of sperm cell in the SST overtime to a level that could not support fertility. On the other hand, it could also be due to the fact that the stored spermatozoa in the oviduct has become stale or aged to achieve fertility since there was no repeated insemination. This study corroborated the work of Tabatabaei *et al.* (2009) who compared fertility in indigenous and Ross chicken and observed Optimum fertility on day 1-7 post insemination and drop to zero on day 19 in indigenous chickens and day 17 for Ross chickens. Bramwell (2002) recorded zero

fertility on day 22 after a single insemination in broiler breeder flock. Brillard (1993) reported that length of fertile period in chickens is 21days. Donoghue and Wishart (2000) indicated that in the absence of frequent copulation or artificial insemination mechanism, slow discharge of sperm cells assured a series of fertilised eggs. As spermatozoa of chicken aged, the ability of the gamete to induce fertilization reduces gradually and this cannot support normal development of embryos. Douard *et al.* (2000) also reported that sperm motility, viability and integrity morphology decreases progressively in storage. From the current study, a strong relationship existed between fertility and days of insemination, with subsequent decline in fertility as day’s post-insemination increases. The significantly lower hatchability observed via natural mating in this study compare to other treatments could be as a result of low fertility recorded in the treatment. This was in agreement with the report of Surai and Wishart (1996) and Hocking and Bernard (1997) and the findings of Robinson (1996) who opined that when artificial insemination is practiced, hatchability parameters are increased compared to natural mating.

5.3: Fertility Trend in Female Broiler Breeder Chickens Inseminated at Varied Intervals with Undiluted Semen for a Period of Fifteen Weeks

Result of first experiment, indicated that semen dose of 0.02ml maintain high fertility level and also economical when compare to other doses and this informed the choice for semen dose and interval used in this experiment in study 2.

Lower fertility observed from 12 days insemination interval implies that 12 days priming dose over time might not prolong fertility comparable to 3, 6 and 9 days insemination frequency. This could probably result from depletion in the storage tubules over time and not sperm age. At the onset of this study sperm storage tubules was filled for 2 consecutive days, but afterwards, a single insemination was done for different insemination frequency. This may be said that the single insemination dose did not substitute for the depletion for the 12days to ensure optimal fertility rate for the hen in insemination frequency of 12 days. This implies that insemination of 12 days interval was not sufficient to fill the sperm storage tubules. From the result observed in study 1 shows that breeder hens can effectively store their spermatozoa for at least 7days, Although, Haque and Hossain (2011), reported two - three weeks.

5.4 Fertility, Hatch Assessment, Embryonic Mortality and Chicks Quality in Female Broiler Breeders

Significantly higher fertility recorded in 3 days interval compared to other treatment could be as a result of repeated insemination intervals compared to other inseminated groups. The result was similar to the findings of Marire (2011) pointing out that insemination of chickens twice weekly could lead to maximal fertility. Significantly better fertility has been reported with weekly insemination in turkey. However, Bratte and Ibe (1989) suggested undiluted semen administered once a week would adequately Sto optimize fertility under tropical conditions. In the present study, 12 days insemination interval reduced fertility and could be indicative of depleted sperm cells in the oviduct with time. Also, 12 days priming dose might not be sufficient to make up for the sperm depleted over time. Embryo death (early and mid) resulted from each groups of insemination frequency, hatchability of eggs and chicks quality was similar among the treatments. Abudabos, (2010) reported that mid embryonic mortality is hardly ever observed only in the case of nutritional condition or abnormality. Late embryonic death observed could be as result of hatchery mishandling, issues with incubator temperature and or poor handling of fertile eggs in the setter during candling at the 18th day.

The significant difference observed in hatch of set in 12 days insemination frequency was due to lower fertility and embryonic mortality recorded in birds on 12 days insemination intervals. It is predictable since the hatchability did not significantly vary between the treatments. Fairchild *et al.* (2002) reported that factors such as fertility of egg and embryonic mortality affect hatchability. Findings of Bramwell (2002) stated that when old sperm cells fertilise an egg, there is likely to be rise in the occurrence of early embryonic death (EED) and chick's quality. This period of time is stated to be 12days in breeder broilers. Embryonic death in chickens could be linked to fertilisation as a result of old sperm cell from the Oviductal storage (Bramwell, 2002). The chick's quality was not affected irrespective of the insemination frequency adopted and likewise the chick's quality was not poorly affected even till 12 days insemination frequency, Value recorded was above 90% of the total chicks hatched for all the treatments. This showed that reducing insemination frequency will reduce stress on the

chickens, safe cost of labour and increase the profit margin of the farmers, if A.I is adopted on least viable semen dose.

5.5 Quality Parameters of Undiluted and Diluted Semen with Different Diluents for Four Hours at Room Temperature (27.1^{O}C to 27.8^{O}C)

In this study, maximum motility was maintained for 3 hours with undiluted semen which contained seminal plasma only to support motility compared with diluted semen (Modified Ringer's and dextrose saline). The diluents utilised in this study have pH and osmolarity range of 6.70 - 6.79 and 310 – 360m Osm/L respectively which is required of a diluent to sustain fertility of sperm cells (Tabatabaei, 2010). In line with this, Adebisi and Ewuola (2019b), reported that to sustain turkey sperm cell *In vivo* osmotic balance is the primary condition and established that spermatozoa motility was maximal within one hour at room temperature with diluted semen. This was in agreement with the result observed in this experiment, with no variability among the groups. Dextrose is an energy source contained in dextrose saline which is probably responsible for sustaining high motility compared to normal saline that contained only sodium chloride that is expected to sustain osmotic stability, this could have been attributed to the reduced sperm motility in the second hour compared to others diluents.

However, modified Ringer's solution does not contain any energy source and is still able to sustain motility just as long as dextrose saline. This can be as a result of reduced sodium chloride in modified Ringer's solution when equated to dextrose and normal saline which might result to a decrease in energy needed to sustain sodium potassium pump in membrane balance leading to more availability of energy for mobility. Reduction in sodium chloride fluid affect energy release by sodium pump in perfused hearts (Bhargava *et al.*, 1964) which makes more energy available for the cell. Potassium chloride present in modified Ringer's solution may make up for sodium chloride in stabilizing membranes as ions of K^- and Cl^+ are also important components of cellular fluids (Nicole, 2015). Ashizawa *et al.*, (1992) observed that calcium chloride in modified Ringers solution improved motility as calcium *in vitro* is known to increase sperm motility.

Spermatozoa motility based on storage time had a high predictability (0.92 to 0.97). The highest gradient of the slope is 13.3 recorded with dextrose saline which implies that the rate of decline in sperm motility in that group was the fastest. The least gradient

of slope was recorded in normal saline (9.2) and undiluted semen (7.2) meaning the rate of drop of sperm motility was slowest.

After the four hour holding period, membrane integrity of plasma and cell liveability remained at maximum, inferring that irrespective of the decline in motility, the structural and functional integrity of cell membrane was maintained. This corroborates the study of Adebisi and Ewuola (2019b) who reported that turkey semen membrane integrity and sperm liveabiity remained at the maximum four hours. Bilgili *et al.* (1987), also reported that at four hours percentage of dead sperm cells was at minimum after collection of semen then after 24 hour at room temperature it increases.

5.6 Egg Fertility and Hatchability from Broiler breeder Hens Inseminated for Two Consecutive Days with Undiluted and Diluted Semen

Regardless of dissimilarity perceived in motility in *in vitro* experiment amongst the diluted (dextrose saline, normal saline and mordified Ringer's solution) and the undiluted semen, fertility response among the treatments was not significant. This means that the diluents are all appropriate for use to dilute semen for insemination purpose in order to achieve good fertility. From this study, it can also be affirmed that what is obtainable *in vivo* is different *in vitro.*

Hens inseminated with different diluted semen at 1:1 dilution ratio had similar fertility up to 14 days post insemination. However, at 21 days post-insemination, relatively higher egg fertility was observed with dextrose saline compared to Normal saline and undiluted semen. This might be as a result of dextrose, an energy source which probably provided energy for the sperm cells for that period of time. This duration was longer in turkeys than chickens according to Adebisi (2018) who observed maximum fertility for up to 6 weeks after a single insemination using different diluent in turkey hens. In addition, in this study, diluent type did not affect hatchability even up to 14 days post- insemination, Donoghue and Wishart (2000) noted that diluents maintained the pH media, osmolarity and made energy available to the sperm, adding that the motility and metabolism in sperm is dependent on pH, as sperm motility declines with reducing pH while a high pH elevate the rate of metabolism. The result of hatchability in this current study corroborates report of Rahim *et al.* (2001) reporting that ideal semen diluents are highly important in applying artificial insemination, so as to preserve semen in optimal state using fresh semen during, artificial insemination show

better results. Diluent constituent example like buffers, osmotic regulators, energy source and antibiotics and so on are necessary in diluent to prevent the spermatozoa against harmful effect *in vivo.*

5.7 Progressive Spermatozoa Motility in Dextrose Saline Diluted Semen of Broiler Breeder Hens at Room Temperature

The lower values observed in progressive spermatozoa motility at the 1st and 2nd hours with higher dilution ratio, corroborate the findings of Adebisi and Ewuola (2019b) who observed a reduced spermatozoa motility in higher dilution ratio at the1^{st} and 2nd hours using normal saline as diluent in turkey semen. Several authors have reported lower spermatozoa motility with higher dilution ratio in chicken semen. Parker and Mc Daniel (2006) reported lower spermatozoa motility with higher dilution ratio of semen with normal saline compared to other diluents. Omprakash *et al* (1992) reported that 1:3 dilution ratio in chicken had higher percentage of spermatozoa abnormality; 1:4 ratio for turkey semen (Sexton, 1987) and 1:2-50 ratio in chickens semen was recorded by (Parker and Mc Daniel, 2006). Higher dilution can result in reduction in the seminal plasma and result in low pH, which in turn can lead to increase in sperm abnormality and also cause reduction in respiration as a result of high dilution ratio. Garner *et al.* (2001) reported that dilution of semen can result to reduction in seminal plasma which can be detrimental to spermatozoa, if the diluent does not serve the function of the seminal plasma after dilution. Low pH reduces motility of sperm and its metabolic rate (Donoghue and Wishart, 2000). Though, the result in this study was not in agreement with the findings of Varner *et al*, (1989) due to the diluents used in both studies. Report of Varner *et al*. (1989) showed increase in spermatozoa motility with high dilution ratio in equine semen using milk Glucose base diluents. The dilution ratio used was 1:1, 1:2, 1:3 and 1:4 stored at 25 ^{O}C (0.5 to 24 hours) was higher than the undiluted semen. The increase in spermatozoa motility might be ascribed to the extender used (glucose skim milk extender) which could have decrease the malformation and detrimental effect that could have arose from dilution of the semen. Thus, to reduce the detrimental effect of dilution ratio on spermatozoa motility, the choice of extender used will be of paramount importance.

Conversely, from the observation in this study dextrose saline with dilution ratio of 1:1 and 1: 2 in broiler breeder cock semen can still be used for insemination within an hour after semen collection.

5.8 Effects of Dilution Ratio and Frequency of Insemination on Fertility of Eggs in Broiler Breeder Hens

Different insemination intervals affected fertility of eggs, as higher fertility was observed in 3 days (75.69%) compared to 6 days (60.59%), but did not differ significantly from 9 days (64.15%). This is expected as a result of the frequency of insemination that was observed in study 2. However, Percentage fertility observed by different dilution ratios was not significantly different. Donogue and Wishart (2000) reported that the ability to obtain fertility results similar to natural mating was dependent on the rate of dilution. Donoghue and Wishart (2000) noted that several factors must be taken into account with spermatozoa which include temperature, energy source, osmotic pressure, electrolyte balance, pH and buffering capacity, microbial control which can affect fertility. Donoghue and Wishart (2000) noted that semen is often diluted in artificial insemination practices to provide a suitable medium that will sustain and protect spermatozoa thus prolonging their fertilizing capacity until they are used for insemination.

The interaction effect of the 2 factors elements was significantly substantial at 3 days frequency. Which denotes that at 9 days frequency, fertility was the same via the dilution ratio, on 6 days intervals 1:1 ratio was notably significantly reduced than 3 days (1:1), but 1:0 was the same to all the groups. The reduction in fertility could be a function of the overall relatively smaller quantity of sperm cells inseminated due to dilution. The findings negate the report of Sexton (1979) stated that inseminating chicken weekly could still produce high fertility at dilution ratio of 1:4, 1:5 and 1:10. This decrease is however only significant when insemination dose contains less sperm cells than the minimum fertility optimizing number. According to Abutu (2011), excess dilution of semen would result in loss of fertility. This can be ameliorated by inseminating chickens weekly even at ratio up to 1:4. Sexton (1987) observed better fertility when stored turkey semen was diluted at 1:1 and 2:1 compared with 1:2.

5.9 Effect of Varyied Ratio of Dilution and Frequency of Insemination on Hatch Variables and Embryo Motality in Broiler Breeder Hens

In this present study, while difference was exhibited in fertility there was no interaction between semen dilution ratio and frequency of insemination and their interaction effect did not show significant difference. Likewise, no variance on hatch of fertile eggs and early embryonic mortality at different stages (early, mid and late) as observed in current study on broiler breeder. The significantly lower hatch of set eggs detected in 6 days insemination interval and the interactions effect 6days (1:1) could be due to the lower fertility observed. Meanwhile, the primary determinants of hatch of set eggs are fertility and embryo death. The report of Hemming and Birkhead (20I5) says that a finite number of spermatozoa should fuse with the ovum to form a zygote and develop. When physiological polyspermy is reduced there is low occurrence of embryo death and this increased survival (Bakst, 1998). There was no significant change in hatchability which is a component of embryo mortality and fertility, which shows that embryo mortality was not affected by the dilution ratios and frequency of insemination. Semen dilution at 1:3 has been reported to increase the percentage of abnormal sperm cells (Omprakash *et al*. 1992). Obvious advantages of semen extension have generated interest in finding dilution rates for semen of avian species.

5.10 Effect of Insemination Frequency and Semen Dilution Ratio on Sperm Recovery at Different Parts of Oviduct and Uterovaginal Junction

In the current study, no significant pattern was evident after washout of spermatozoa from the oviduct, relating to dilution ratio of semen nor frequency of insemination. From the current study, infundibulum sperm count was not significantly affected by dilution ratio and storage intervals but sperm count in magnum/isthmus and uterus/ vagina was influenced by insemination intervals. As sperm count in magnum /isthmus was significantly higher in 3 days (1.07) and 6 days of interval (1.10), compared to 9 days (0.66). This shows that other than the frequency of insemination or number of sperm cell inseminated, other fundamental factors are liable for the spermatozoa number that occur in the oviduct at all time. During fertilization spermatozoa are released from the sperm storage tubules which were under some unidentified stimulation associated with follicular maturation (Bakst and Bird, 1987). Ova position in the oviduct at time of laying are added factors Howarth (1971) An egg at the

magnum,- isthmus and proximal part of uterus can serve as an obstacles affecting the releases of spermatozoa from Sperm storage tubules in passage to the sperm storage site. Higher number of spermatozoa was found in the oviduct without an ovum compare with the one with ovum as reported by (Bakst, 1981). The presence of spermatozoa were found in almost all the regions of the oviduct. This supposes that the uterovaginal gland releases sperm cells continuously and not just at ovulation. Also, it was observed in the current study that sperm recovery from the infundibulum and magnum/isthmus were proportionally lower compared to those recovered from the uterus/vagina segment. This may be expected because the site (infundibulum) with relatively lower sperm cell count is the secondary host for Oviductal sperm storage. Although, the utero /vagina is the primary host of oviductal sperm storage while the infundibulum is the secondary host (Bakst, 1998).

The outcome concentration of spermatozoa at the Uterovaginal junction which is the sperm storage tubule of sperm cells exhibited a direct increase with insemination frequency and sperm quantity inseminated. By indication, this means that the higher the number of sperm cells inseminated, the higher the number of cells released to the sperm storage tubules. The effect of this is that inspite of the disparities at different part of the oviduct; the release of the sperm cell from the sperm storage tubules is similar even with variance in the number of cells inseminated; what is left in the uterovigina junction, is a function of the number of sperm cell inseminated. This shows the utero-vagina as the primary site of stored sperm cells in the oviduct of the hen (Bakst, 1998). Consequently, sperm cells number at the uteroviginal junction is dependent on quantity of spermatozoa inseminated into the oviduct.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 SUMMARY

Period of efficient fertility was eight days in broiler breeder hens, while maximum duration was twenty – one (22days) irrespective of the semen dose administered. Inseminating undiluted 0.02 mL semen containing (5.6 million sperm cells) maintained fertility level similar with the higher doses.
Broiler breeder hens under natural mating inseminated for two consecutive days gave a lower average fertility result of 76.9% compare the inseminated group with over 90% fertility for the first 7 days post insemination.

In broiler breeder hens, embryo mortality was not affected irrespective of the insemination dosage administered and the mating type.

An insemination interval of 9 days with 0.02ml semen gave similar fertility result (76.3%) with 3 days (80.4%) and 6 days (74.3%) over 15 weeks, while 12days frequency resulted to reduced fertility (67.8%).

It was observed that hatchability and chick quality were not affected by insemination frequency of 3, 6, 9 and 12 days with values ranging from 79.16% to 82.74% and 90.38% to 96.54% respectively.

Integrity of plasma membrane and liveability of sperm cells were maintained up to 4 hours in undiluted and diluted semen stored at room temperature.

Utilizing modified Ringer's solution or 1% dextrose saline at ratio 1:1 as semen diluent did not improve sperm cell motility (61.7% and 58.3%, respectively) after 3 hours *in vitro* compared with 0.9% saline diluted semen (1:1) and undiluted semen with higher motility. However higher fertility was maintained for 1 week post insemination for the undiluted, normal saline, dextrose saline and Ringer's solution diluted semen and zero fertility was reached by all groups at 4weeks.

Different dilution ratios did not significantly affect fertility in broiler breeder hens. However, 3 days insemination interval enhanced fertility compared to 6 and 9 days.

Semen dilution ratio and insemination frequency did not influence embryonic mortality.

There was no definite pattern in the number of sperm cells at different segment of the oviduct across the treatment groups, however, the sperm cells in the uterus/vagina segment was relatively higher than those at the infundibulum and magnum/isthmus.

6.2 CONCLUSION

In the adoption of artificial insemination in Marshall's broiler breeder hens, undiluted semen (0.02mL containing 5.6 million sperm cell) using up to 9 days insemination frequency was enough to maintain optimum fertility more than 15weeks with no detrimental effect on hatchability and phenotypic quality of chick's

Normal saline and 1% dextrose saline were suitable diluents for artificial insemination in Marshall's broiler breeder chickens.

Different dilution ratios did not significantly affect fertility in Marshall's broiler breeder hens. However, 3 days insemination interval gave similar fertility as 9 days.

6.3 RECOMMENDATIONS

The Marshall's breed of broiler breeder hens examined in the current study has shown improved fertility response and high hatchability prospect under tropical conditions using artificial insemination technique. Nevertheless, further research could be conducted to examine the influence and interactions of breeds and seasons on quality of semen, fertility and hatchability of eggs in broiler breeder hens reared in the tropics under artificial insemination.

Also, further bio-compounds with effective anti-microbiological importance could be included in semen diluents for semen shelf life extension for on-farm use.

6.4 CONTRIBUTIONS TO KNOWLEDGE

(1) Semen dose of 0.02mL (5 million motile sperm cells) can sustain maximum fertility of about 94% for 7 days without repeated insemination.

(2) The period of efficient duration of fertility was 8 days, while maximum duration was 22days for artificially inseminated hens and 19 days for naturally mated hens.

(3) Insemination intervals of 9 days could sustain fertility in Marshall's broiler breeder hens without any compromise on embryo viability and chicks' quality.

(4) Integrity of plasma membrane and liveability of sperm cell stored at room temperature can be maintained up to 4 hours in diluted and undiluted semen.

(5) Both dextrose and normal saline were appropriate as semen diluents for on-farm insemination in Marshall's broiler breeder hens.

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