IMPROVEMENT OF REPRODUCTIVE PERFORMANCE OF RABBIT DOES BY HORMONAL AND ACETIC ACID TREATMENT IN HOT-HUMID CLIMATIC CONDITION OF BANGLADESH

A THESIS

BY

MD. HOSNE MOBARAK

Registration No.: 1305095

Session: 2013-2014

Semester: July-December, 2014

MASTER OF SCIENCE (M S)

IN

GENETICS AND ANIMAL BREEDING

DEPARTMENT OF GENETICS AND ANIMAL BREEDING

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Submitted to the Department of Genetics and Animal Breeding, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur for partial fulfillment of the requirement of the degree

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December, 2014

Dedicated to.........

My father who insisted me the value of my education, my mother whose unending love and sacrifices inspired and encouraged me and the Almighty Allah who blessed me with the ability and strength to accomplish it.

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The Author

Abstract

This study was carried out with New Zealand (White, Black and Brown) rabbit to improve the reproductive efficiency of does using hormonal and acetic intra-vaginal washing treatments. Three experiments were conducted from March to September, 2014 in the Rabbit Farm under the Department of Genetics and Animal Breeding at Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh. Under the experiments the effect of prostaglandin ($PGF_2\alpha$) injection, intra-vaginal washing with acetic acid and combination of prostaglandin ($PGF_2\alpha$) and Gonadotropin Releasing Hormone (GnRH) injection were observed. The traits: conception rate, gestation period, litter size (at birth and weaning), litter weight (at birth, at 7, 14, 21 and 28 days of age), individual kit weight (at birth, at 7, 14, 21 and 28 days of age), mortality rate of kits, live weight gain of rabbit does during the experimental periods were evaluated and post mortem examination of productive and reproductive organs of low fertile and infertile rabbit does were performed. $PGF_2\alpha$ (supplied by Techno Drugs Ltd. Bangladesh) injection did not affect conception rate, gestation period, litter size at birth and weaning, litter weight from birth to weaning, individual kit weight from birth to weaning, kit mortality, doe live weight gain. Intra-vaginal washing with acetic acid increased ($P<0.05$) conception rate, litter size at birth, litter weight from birth to weaning, kit mortality, doe live weight gain. However, the combined use of $PGF_2\alpha$ and GnRH (Bayer, New Zealand) increased ($P<0.05$) conception rate, litter size, litter weight from birth to weaning, individual kit weight from birth to weaning, kit mortality, doe live weight gain.

Key Words: Doe, conception rate, gestation period, gonadotropin, kit mortality, litter size, prostaglandin, rabbit and reproductive performance.

Abbreviation and Acronyms

BBS	Bangladesh Bureau of Statistics
BP	British Pharmaceuticals
CRD	Completely Randomized Design
CP	Crude Protein
DLS	Department of Livestock Services
FAO	Food and Agricultural Organization
FC	Feed Consumption
FCR	Feed Conversion Ratio
FSH	Follicle-Stimulating Hormone
GDP	Gross Domestic Product
GNI	Gross National Income
GnRH	Gonadotropin Releasing Hormone
IRRG	International Rabbit Reproduction Group
Kcal	Kilocalorie
LH	Luteinizing Hormone
ml	Milliliter
ng	Nanogram
NaCl	Sodium chloride
NRC	National Research Council
$PGF_2\alpha$	Prostaglandin
PMSG	Pregnant Mare Serum Gonadotropin
SPSS	Statistical Package for the Social Sciences
SID	Statistics and Informatics Division
UNFPA	United Nations Fund for Population Activities
µg	Microgram

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Chapter I

Introduction

Bangladesh is a densely populated agriculture based country with 154 million people and positioned currently 8th among all the countries of the world (UNFPA, 2012). The human population growth in developed countries is stabilizing while that of developing countries including Bangladesh is increasing rapidly. According to the World Bank information (2012), Bangladesh ranked 61st (in 2011) in respect of human poverty. The depth and severity of poverty is worse in rural areas, 80% of the total population lives in the rural areas that primarily depend on a poorly developed agriculture for livelihood (BBS, 2014). The domestic production of milk, meat and egg are 3.46, 2.33, and 7303 million tons in the 2011-2012 fiscal year against the demand of 13.50, 6.48, and 15392 million tons, respectively (Hossain, and Hassan, 2013). There is a deficit of milk (80%), meat (82%) and eggs (63%), (FAO, 2008). Due to the severe poverty most of these people faces an acute deficiency of animal protein sources, like meat, milk, egg etc. Among the livestock sector, the poultry industry was becoming a leading industry in our country. But unfortunately, the industry is in the line to be destroyed due to severity of avian influenza (bird flu). Thus, it is crying need to search the alternative protein source to meet up the increasing demand.

In order to maximize food production and meet protein requirements in developing countries, variable options need to be explored and evaluated (Owen *et al*., 2008). Among such alternatives, the use of livestock species that are yet to play a vital role in production of animal protein is very important. Fast-growing livestock such as, rabbit possess a number of features that might be of advantages in the small holder farming system in developing countries. The rabbit has immense potentials and good attributes which include high growth rate, high efficiency in converting forage to meat, short gestation period and high prolificacy, relatively low cost of production, high nutritional quality of meat which includes low fat, sodium, and cholesterol levels. It also has a high protein level of about 20.8% and its consumption is bereft of cultural and religious biases (Biobaku and Oguntona, 1997). The presence of caecal microbes enables the rabbit to digest large amounts of fibrous feed as most non-ruminant species can not (Taiwo *et al*., 1999). Their by-products serve as major diet components and are devoid of fat thus making them important source of protein. The manure can be used as fertilizer for crops and gardens.

Rabbit production is a growing development in this region, which plays an important role in view of the economic risks by the spread of Asian bird flu (Otte *et al*., 2007). According to the FAO (2001), backyard rabbit keeping provides additional income and supplies additional protein for poor rural and urban households with low investment and labor inputs. However, the major limitation to the development of small-scale rabbit production is the adverse effect of heat stress on reproductive performances during summer season of Bangladesh. Rabbits do not cope well in temperatures above 32.2°C and in high humidity. The rabbits can withstand cold weather than warmer one. Their fur coat does not easily allow them to radiate body heat. Ideal temperatures for rabbits range from 10 to 15.6°C. Heat stress occurs whenever an animal has excess body heat that it can not lose. In Bangladesh, high temperatures during the summer, is a major constraint factor for rabbit production, as it negatively affects production. Rabbits are highly susceptible to heat stress due to few functional sweat glands and evince difficulty in heat evaporation with increasing environmental temperature (Cheeke *et al*., 1987). There is evidence that high ambient temperature can impair the reproductive performance of rabbits. Heat stress can cause sterility among bucks. As a result of heat, does may not reproduce. Instead they could miscarry, abort their young, ignore the newborn, or deliver outside the nest box on the wire floor. Rabbits also can die from heat stress that can result in high mortality rates.

Many attempts have been done to overcome the adverse effects of heat stress by modifying environmental condition through nutritional, managerial, and physiological manipulation of rabbits (Selim *et al*., 2003). Prostaglandins are a group of endogenously occurring acidic lipids that involved in a large number of reproductive processes. In domestic animals, the most important and the practical utility of prostaglandin appears to be $PGF_2\alpha$ (Lauderdale, 2002). Synthetic prostaglandin analogues are molecules which are manufactured to bind to a prostaglandin receptor. Most prostaglandins act at or near the site of production and can affect a variety of reproductive functions including ovulation, fertilization, implantation, embryonic development, luteolysis, parturition and lactogenesis (McNitt, 1992). In rabbits, ovulation is a neuroendocrine reflex that is physiologically induced at the condition of natural breeding.

The mechanisms whereby rabbit does become spontaneous ovulators are still unclear, but are likely associated to factors interfering with the control of the gonadal axis involving the hypothalamic centers responsible for GnRH release (Theau-Clement *et al*., 2008). Ovulation can be induced artificially in does by intramuscular injection of either Gonadotropin-releasing Hormone (GnRH), or its synthetic analogues (Moce *et al*., 2003; Rommers *et al*., 2004; Quintela *et al*., 2004). Highly potent, long-acting synthetic analogue of GnRH have been produced and is available commercially (e.g., gonadoreline, buserelin, fertirelin acetate, desloreline and fertagyl). These compounds proved a new tool for inducing endogenous Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH) release and induce ovulation in all mammalian species (Taponen *et al*., 2003). In this concern, Bonanno *et al*. (2008) induced ovulation in rabbits at artificial insemination by intramuscular injection of 0.8 μg of synthetic GnRH (buserelin).

Actually all managerial initiatives like using air condition, fan, cooled water bottle in cage, water spray were not sustainable in case of small-scale rabbit production. Ultimately, nutritional, hormonal and physiological manipulation may shout to investigate to overcome the adverse effects of heat stress. One study suggested that the diet containing 21% CP improved the productive and reproductive performance of rabbit under hot climatic condition of Bangladesh (Salma *et al*., 2004). The treatments of the females with $PGF_2\alpha$ and GnRH are commonly used to improve reproductive performance, through maturation of ovarian follicles and sexual receptivity (Maertens *et al*., 1995). Among the physiological manipulation of rabbits, acetic intra-vaginal washing treatment attributes to increase blood flow and develop vulva color which led to increase mating acceptance and possibility of conception (Gounis *et al*., 2002)

However, there was no systemic study with the improvement of reproductive performances of heat-stressed does during summer season of Bangladesh. So, the adverse effects of heat stress especially on reproductive performance were hypothesized to be overcome by hormonal treatments and intravaginal washing with acetic acid. Therefore, the present study was designed with the following objectives:

i) To know the effect of hormones ($PGF_2\alpha$ and GnRH) and acetic intra-vaginal washing on reproductive performance of rabbit in hot-humid climatic condition of Bangladesh.

ii) To improve the reproductive performance of low fertile or infertile rabbit does.

Chapter II

Review of Literature

This chapter presents the review of relevant literatures, which consist of the effects of heat stress, hormones and acetic acid on productive and reproductive performance of rabbit. Many researchers have been conducted researches in these topics. But in Bangladesh, limited research work has been performed. The reproductive success depends on culminating in the delivery of viable fetuses followed by nursing of offspring, necessarily requires tight co-ordination and the fine-tuning of various sequential processes encompassing follicular development, ovulation, fertilization, embryogenesis, embryo implantation and gestation. The improvement and homogenization of reproductive performance on farms are conditioned by the use of methods enabling the induction and synchronization of oestrus. This concerns hormonal treatments or non-hormonal alternative methods. Hormonal treatments have been widely used in recent years. With these treatments, different types and dosages of hormones are administered before insemination.

2.1. Effects of heat stress on rabbit

The endocrine system plays an integral part in the animal's response to stress (Ayyat *et al*., 2004). High temperatures, as encountered in Bangladesh and in many other countries during the summer, is a major constraint factor for rabbit production, as it negatively affects production due to heat stress (Fouad, 2005). Rabbits are highly susceptible to heat stress due to few functional sweat glands and evince difficulty in heat evaporation with increasing environmental temperature (Cheeke *et al*., 1987). The metabolic rate increased about 20% in rabbits when exposed to high air temperature ranged form 30 to 35°C while, the feed intake was decreased (Habeeb *et al*., 1993).

In New Zealand White rabbit, temperature of 32.8°C reduced fertility in the male rabbits, a continuous high temperature being more detrimental than intermittent heat (Oloufa *et al*., 1951). Temperatures around or above 30°C cause drop of quantity and quality of semen (Bicudo and Paschoal, 1991; EL-Masry *et al*., 1994; Finzi and Macchioni, 1994; Viudes-de-castro and Vicente, 1997). The hot climate negatively affects the quality of oocytes and the process leading to the correct formation of meiotic chromosomes, as represented by the decreasing of telophase I and metaphase II (Hamam *et al*., 2001). In addition, Al-Katanani *et* *al*. (2002) reported that the proportion of oocytes and cleaved embryos that developed to blastocysts was lower in the warm season than in the cool season. Also, short exposure of pre-implantation rabbit embryos to elevated temperatures (41.5ºC and 42.5ºC) *in vitro* reduced embryo development (Makarevich *et al*., 2006).

2.2. Effect of Prostaglandin ($PGF_2\alpha$) and Gonadotropin-Releasing Hormone (GnRH) in reproductive performance of rabbit does

The whole reproductive procedure is under hormonal control, but interactions with the other two main regulatory systems of the organism, the nervous and immune systems, also occur as exemplified by the neurocrine nature of the hypothalamic hormones sub serving the pituitary gland and by the dual role of the prostaglandins acting either as hormone or mediator of the inflammatory response (Boiti *et al*., 2006).

Prostaglandins are structurally classified into nine major groups, A to I to indicate the type of substituent found on the hydrocarbon skeleton and containing subgroups denoted by the subscripts 1, 2 and 3 to indicate the number of double bonds in the hydrocarbon skeleton (Oliw *et al.*, 1983 and Parker and Parker, 2004). In domestic animals, the most important and the practical utility of prostaglandin appears to be $PGF_2\alpha$ (Lauderdale, 2002). The prostaglandins are found in most tissues and organs. They are produced by all nucleated cells except lymphocytes. They are autocrine and paracrine lipid mediators that act upon platelets, endothelium, uterine and mast cells. They are synthesized in the cell from the essential fatty acids (Laneuville, 2003). Synthetic prostaglandin analogues are molecules which are manufactured to bind to a prostaglandin receptor. These molecules are classically associated with sites of inflammation and thus their presence in ovulating follicles lends credence to the idea that ovulation is an inflammatory like process (Espey and Richards, 2006).

The relationships between female sexual behavior, sex steroid hormones, lactation and color and turgidity of the vulva have been investigated in rabbits throughout pregnancy, pseudopregnancy, and the post partum (Rodriguez and Ubilla, 1988; Stoufflet and Caillol, 1988 and Theau-Clément and Roustan, 1992). Prostaglandin $F_2\alpha$ injection could have very rapid and dramatic effects on steroid synthesis in the lutein cell whereas normal luteolysis would seem to involve more gradual regression of the gland. This may have some relevance in explaining the mating acceptance post-injection $PGF_2\alpha$.

Many studies (Bourdillon *et al*., 1992; Theau-Clément and Lebas, 1994; Davoust *et al*., 1994) concur that multiparous does generally show good performance (high fertility rate, medium litter size). Oestrus synchronization by means of PMSG injection two or three days before insemination has become very common in industrial management, as this practice generally improves reproductive performance (Bonanno *et al*., 1991; Castellini *et al*., 1991; Bourdillon *et al*., 1992; Angeli *et al*., 1990; Gogol, 2004). Zapletal *et al*. (1990) reported that the total annual fertility rate was higher (73.02%, $P<0.05$) in rabbit group treated with lecirelin (GnRH). It has been shown that GnRH mediates the hypothalamic control of pituitary gonadotropin secretion and biosynthesis (Dekel *et al*., 1988) to induce ovulation. However, the obtained improvement in kindling rate and litter size of rabbit does treated with GnRH as compared to the controls in this study was also due to the effects of GnRH on the ovary include stimulation of oocyte maturation (Dekel *et al*., 1988; Yang *et al*., 1995).

In addition, GnRH may interfere with the stimulatory effects of exogenous gonadotropin on follicular development, corpus luteum establishment and oocyte maturation (Zanagnolo *et al*., 1996). Some studies have shown that GnRH and its analogues also exert a direct effect on gonadal function, influencing rabbit oocyte maturation both *in vivo* and *in vitro* (Yang *et al*., 1995; Yoshimura *et al*., 1992). It has been suggested that GnRH induces oocyte maturation via activation of specific GnRH receptors on granulosa cells (Koves *et al*., 1989). Exposure to GnRH stimulates prostaglandin synthesis in pre-ovulatory follicles (Wong and Richards, 1992).

2.2.1. Sexual receptivity

Rabbits do not have a normal oestrous cycle like bovines and they are often, erroneously, considered to be permanently in oestrous. Sexual receptivity can be evaluated more accurately by the color of the vulva (IRRG, 2005). The relationships between female sexual behavior, sex steroid hormones, lactation and color and turgidity of the vulva were investigated in rabbits throughout pregnancy, pseudopregnancy, and the post partum (Rodriguez and Ubilla, 1988; Stoufflet and Caillol, 1988 and Theau-Clément and Roustan, 1992). In the post partum period, the does are highly receptive the first day after parturition and shortly (1-2 days) after weaning (Theau-Clement *et al*., 2000). However, great variability exists among rabbit females depending on parity, lactation stage, and other factors (Theau-Clément and Roustan, 1992).

Dragan *et al.* (1996) suggested that sexual receptive was better from 85 to 95% of does treated with prostaglandin analogues compared to the controls (67.5). Hassanein (2000) found that the percentage of mating acceptance was 100% when administering 2.5 mg of Lutalyse® ($PGF_2\alpha$ analogue) before natural mating with fertile bucks. Theau-Clement and Roustan (1992) found relation between receptivity and each of ovulation or fertilization.

Many authors (Mills and Gerardot, 1984; Rodriguez *et al*., 1989; Rebollar *et al*., 1997) observed that the highest LH and FSH concentrations reached after 60-90 min exogenous administration of GnRH, decreasing to basal concentrations at 4-5 h later. Muelas *et al*. (2008) did not find change in FSH concentration before or after 48 h of mating and 2 h after mating. Also, Ubilla *et al*. (2000) did not find any difference in FSH concentrations between 48 h before and 7 h after of artificial insemination. However, the present trend of change in LH was comparable with that observation on rabbits by Muelas *et al*. (2008), who found significant differences among LH plasma concentrations measured before or after 48 h of the mating (5.1 and 5.4 ng ml^{-1}, respectively) vs. 2 h after the mating (29.0 ng mL^{-1}).

In accordance with the marked increase in LH concentration in GnRH-treated does, Rodriguez *et al*. (1989) showed that LH level sharply increased after 15 min of GnRH challenge, being 200-1000%, depending on sexual receptivity of does and GnRH dose. However, Muelas *et al*. (2008) found that the LH levels were 4-6 fold higher after 2 h of the mating in does with high ovulation rate, which may explain the highest litter size of GnRH-treated does as compared to the control group. In addition, this may explain the higher ovarian stimulation and pituitary responsiveness after GnRH biostimulation.

2.2.2. Gestation period

Gestation length in different breed of rabbits observed by Morimoto (2009). Rashwan *et al*. (2003) found kindling date 31-33 days after the mating or artificial insemination. Induction of parturition using prostaglandins is a valuable aid in rabbit management. It allows immediate cross fostering from large to small litters and facilities supervision of does during parturition (Pimenta *et al*., 1996). Inducing parturition of does by synthetic prostaglandin analogue (cloprostenol) injection intramuscularly on day 28 or 29 of pregnancy significantly reduced the length of pregnancy when compared with untreated does (McNitt *et al*., 1997 and Lavara *et al*., 2002). However, Dorra *et al*. (1997) found that, when $PGF_2\alpha$ analogue (cloprostenol) was injected intrauterine after just mating, the gestation period was not affected.

2.2.3. Litter size

The litter size at birth improved by $PGF_2\alpha$ injection (Kirrella *et al*., 1995; Dorra *et al*., 1997; McNitt *et al*., 1997 and Gogol, 2009). Moreover, Kirrella *et al*. (1995) and Dorra *et al*. (1997) reported that the intrauterine injection by $PGF_2\alpha$ might active the uterine contraction, which lead to the increase of sperm number and hence ova fertilization. Abd El-Glil (1993) reported that when does mated within one hour or 48-72 h after $PGF_2\alpha$ injection, the litter size at birth and at 21 days of normal fertile or infertile does were improved. On the other hand, Lavara *et al*. (2002) observed no significant differences in litter size at birth and live-born kits when used synthetic $PGF_2\alpha$ analogue (cloprostenol) compared to control. Also, Pimenta *et al*. (1996) obtained the same results with using natural $PGF_2\alpha$. Some author says, GnRH treatment increased number of kits per litter and decreased kit weight at birth, which is in good agreement with the finding of Zapletal and Pavlik (2008), who reported that the conception rates ranged from 10.0 to 89.5% for different GnRH doses.

2.2.4. Litter weight

Abd El-Glil (1993) revealed that $PGF_2\alpha$ treatment affected the litter weight at birth after does received 100 µg $PGF_2\alpha$ and mated within one hour post-injection, or 48-72 h post-injection and control does, respectively. In addition, $PGF_2\alpha$ treatments increased litter weight at 21 days of age and concluded that there was positive correlation between litter size and weight at 21 days of age. Dorra *et al*. (1997) reported that $PGF_2\alpha$ caused increasing the litter size at birth which in turn increased litter weight at birth. However, McNitt *et al*. (1997) and Hassanein (2000) found higher litter weight at birth of does which treated with saline (as a control group) compared to the does of $PGF_2\alpha$ treatment. However, Lavara *et al*. (2002) observed that litter size at birth was decreased by $PGF_2\alpha$ treatment of does, but weaning weight not affected by the treatment.

2.2.5. Kit mortality

Percentage of mortality was affected in $PGF_2\alpha$ treated groups compared to the control (Hassanein, 2000). According to McNitt *et al*. (1997), does treated with $PGF_2\alpha$ analogue, cloprostenol and saline had intermediate numbers of live kits born. But, Abd El-Glil (1993) and Dorra *et al*. (1997) reported that there was no effect of $PGF_2\alpha$ treatment on viability. However, mortality rate was not affected significantly by GnRH treatment (Ondruska *et al*., 2008). In this respect, Quintela *et al*. (2004) used GnRH-buserelin in considerable higher concentrations for intravaginal use (10 up to 20 times) compared with intramuscular control (0.8 μg per female).

2.3. Effect of intra-vaginal washing with acetic acid in rabbit does

The aim of this part of study was to apply a new non- hormonal method, acetic intra-vaginal washing, to improve and homogenize reproductive performances avoiding, as far as possible, negative effects on young growth and animal welfare through inducing rabbit does sexual receptivity. The rabbit, having a vaginal mucosa more sensitive than that of the rat, remains an appropriate model for measuring the potential irritancy of new douche products, because comparisons can be made with products of known irritancy. Because of the apparent importance of vaginal acidity as a factor predisposing the human vagina to colonization by certain bacterial species, the pH values of rabbit vagina were ranged from 6.6 to 8.1 and the microorganisms recovered from rabbit vagina were always relatively homogenous and small number of species (Jacques *et al*., 1986).

Chapter III

Materials and methods

3.1. Experimental site and animals

This study was conducted with three experiments from March to September, 2014 in the Rabbit Farm under the Department of Genetics and Animal Breeding at Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh. A total of 40 rabbit does (7-8 months of age and 1.5-2.5 kg live weight) as well as 10 fertile rabbit bucks (7-8 months of age and 2-2.5 kg live weight) were used in this study. The females were healthy and in different "physiological states" defined by the combination of parity and state of lactation (nulliparous and multiparous-lactating).

Photo 1. Rabbit house

3.2. Temperature and humidity

The average temperature during the experimental period ranged from 28°C in April to 29°C in August, while relative humidity ranged from 66% in April to 82% in August. Sometimes the temperature and relative humidity were reached up to 37°C and 90% respectively (Table 1).

Table 1: Temperature and humidity at Dinajpur District during March to September, 2014.

Month	Temperature	Humidity
March	15°C	62%
April	28°C	66%
May	29°C	69%
June	29°C	76%
July	30°C	81%
August	29°C	82%
September	28°C	79%

(Source: http://climatevo.com/2014,dinajpur,bd)

3.3. General management

Each breeding animal was identified by ear-marking (Photo 2). All does and bucks were kept individually in flat bamboo-wooden cages (44×64 cm) equipped with waterer and feeder. Experimental does and bucks were kept under the same management. Controlled light-dark cycles (16 h light: 8 h dark) were applied. Cages were cleaned and disinfected every week regularly. Urine and feces dropped from the cages on the floor were cleaned every day in the morning. To reduce heat stroke, 1ml lemon juice was mixed with per liter water when the temperature was very high. On the day of mating, receptive does were transferred to the cage of buck for two hours and observed their mating behavior. After mating, the does were returned back to their own cages. The mating was performed at very early in the morning. For confirmation of conception, the does were mated again by the same buck in the next day. At the 14th day of pregnancy, diagnosis was performed by palpation and live weights of does were recorded (Photo 9).

On day 27 of mating, the nest boxes were provided with soft straw to help the doe in preparing a worm comfortable nest for the kits (Photo 3). In case of all experiments, live weight of does at 28 day after breeding was recorded to calculate the doe live weight gain during pregnancy. After birth of kits, litter size and weight at each week was recorded (Photo 12-15). If litter size was more than six, fostering was performed to help them feeding (Photo 16). Close observation and careful attention were done during the experimental periods to check the mortality and find out any disease problem.

3.4. Feeding management

Does were fed a balanced diet (as basal diet), to meet the nutritional requirement for pregnancy and lactation according to the quantities recommended in NRC, 1977, @80 g/head containing 2750 kcal/kg digestible energy, 17% protein, 4% fat, 2% calcium, and 0.4% phosphorus with 200 g Napier grass per head per day (Photo 5). Water was provided *ad libitum* all over the experimental periods. The required feed (both green grass and concentrate) were offered to all rabbits twice daily, once in the morning at 8.00 a.m. and afternoon at 3.00 p.m.

Photo 2. Ear marking and record keeping

Photo 3. Nest boxes with soft straw

Photo 4. Cultivation of Napier grass for rabbits

Photo 5. Feed, water and grass supply to rabbits

3.5. Experimental design

3.5.1. *Experiment I*

Experiment I was conducted from March to April, 2014. A total of 40 rabbit does were collected from different sources of Dinajpur District and randomly allotted into two equal groups (T_0 and T_1), 20 does were in each group. Each doe of control group (T_0), injected intramuscularly with 1 ml saline solution (0.9% NaCl). Another 20 does were in treatment group (T_1), injected intramuscularly with 0.25 ml Dinoprost® (a Prostaglandin $F_2\alpha$ analogue, Techno Drugs, Bangladesh) at first day of experiment (according to Abdel-Azeem, 2010). Does were mated naturally by fertile bucks at 72 h post-injection of prostaglandin.

Photo 6. Injection of hormone

Photo 7. Intra-vaginal washing with acetic acid

3.5.2. *Experiment II*

Experiment II was conducted from May to July, 2014 after completion of *Experiment I*. A total of 36 rabbit does were randomly assigned into two equal groups (T_0 and T_1), 18 does were in each group. Does of control group (T_0) were intra-vaginally washed with 1 ml saline solution, (0.9% NaCl) and another 18 does (T_1) were intra-vaginally washed with 1 ml glacial acetic acid (1%) by using insulin syringe without a needle. All the does were mated just after treatment with normal saline and glacial acetic acid.

3.5.3. *Experiment III*

Experiment III was conducted from August to September, 2014. A total of 40 rabbit does (low fertile and infertile) were randomly assigned into four groups, containing 14, 14, 6 and 6 does, respectively for control and treatment of low fertile and infertile groups.

In case of fertile rabbits, 14 does were in control group (T_0) (injected intramuscularly with 1 ml saline solution, 0.9% NaCl). Another 14 does (T_1) were injected intramuscularly with 0.25 ml Ovuprost® (each ml containing 250 µg Cloprostenol), a synthetic analogue of prostaglandin $F_2\alpha$ ($PGF_2\alpha$; Bayer, New Zealand) at first day of experiment (according to Abdel-Azeem, 2010). At 72 h post-injection of prostaglandin, 0.20 ml of Ovurelin® (each ml containing 100 µg of GnRH; Bayer, New Zealand) was applied intramuscularly.

A total of 12 infertile rabbits (which were not conceived in first two experiments) were randomly divided in two groups, control (T_0If) and treatment (T_1If) with 6 does each. The does of control group (T_0If) were injected intramuscularly with 1 ml saline solution. The does were under treatment group (T_1If) injected with 0.25 ml Ovuprost® at first day of experiment. After 72 h of prostaglandin injection, 0.20 ml of Ovurelin® was injected intramuscularly. Just after treatment, natural mating was performed by fertile bucks (Photo 10).

3.6. Record keeping

The data related with the reproductive performance of the rabbit does were recorded regularly. Within 12 h after kindling of each doe, litter size and litter weight of live kits were recorded. Doe live weight gain, feed consumption (FC), feed conversion ratio (FCR), length of gestation period (day), conception rate, litter size and weight at birth, 7, 14, 21 and 28 day of age were recorded.

3.6.1. The parameters of does were calculated under the following captions

3.6.1.1. Live weight gain of Does (LWGD)

It was calculated at 28 days after mating by using the following formula:

$$LWGD_{28} = LWD_{28} – LWD_{0}$$

Where:

LWD_0 = Initial weight of does at the time of start of experiment.

LWD_{28} = Final weight of does at 28 days after breeding.

The process of measuring live weight of does were shown in Photo (8).

3.6.1.2. Feed consumption (FC)

Feed consumption per doe was calculated for each doe by sum of the daily consumption for 28 days of age from 1st day of mating.

3.6.1.3. Feed conversion ratio (FCR)

It was calculated using the following formula (according to Ensmingar, 1980):

$$FCR = \frac{\text{Total weight gain (g)/doe during a certain period}}{\text{Feed Consumption (g) / doe during the same period}}$$

3.6.1.4. Gestation period

The duration of pregnancy is also called the gestation period. The gestation period was recorded for each doe during the experimental periods from March to September 2014.

3.6.1.5. Conception rate

The conception rate was calculated for each treatment by the following equation

$$\text{Conception rate (\%)} = \frac{\text{Number of pregnant does}}{\text{Number of mated does}} \times 100$$

Photo 8. Weighing of rabbit doe

Photo 9. Pregnancy diagnosis by pulpation method

Photo 10. Natural mating

Photo 11. Making nest by doe with her own fur before kindling

3.6.2. The parameters of offspring

3.6.2.1. Litter size

The litter size (total, alive and dead) was recorded at birth and at each week up to weaning.

3.6.2.2. Litter weight and individual kit weight

The litter weight and individual kit weight were recorded with the help of digital balance at birth, 7, 14, 21 and 28 days of age. The process of measuring litter weight was shown in Photo (12).

3.6.2.3. Kit mortality

The mortality was recorded up to weaning. The formula of identifying the mortality rate of offspring-

$$\text{Kit mortality (\%)} = \frac{\text{(Total litter size at birth - alive kits number at weaning)}}{\text{Total litter size at birth}} \times 100$$

Photo 12. Weighing of new-born kits

Photo 13. Weighing of kits at 7 days of age

Photo 14. Weighing of kits at 14 and 21 days of age

Photo 15. A group of weaning kits with doe

Photo 16. Fostering of kits

3.7. Vaccination and medication

Prior to the starting of all experiments, animals were treated with @ 0.1ml A-Mectin® (each ml containing 10 mg Ivermectin BP, The ACME Laboratories Ltd., Bangladesh) for prevention of ecto and endo-parasites (Rai, 1988).

3.8. Statistical analysis

The CRD was used to design the experiment. The collected data under this study were analyzed and presented using simple statistical techniques. The raw data were entered and sorted into MS Excel spread sheet, then analyzed using analytical software Statistical Package for the Social Sciences (SPSS, version 16) for descriptive analysis.

$$\mathbf{Y_{ijk} = \mu + BW_i + LS_j + (BW \times LS)_{ij} + e_{ijk}}$$

Where,

Y_{ijk} = data of individual animals

μ = overall mean

BW_i = effect of birth weight

LS_j = effect of litter size

$(BW \times LS)_{ij}$ = interaction of birth weight and litter size and

e_{ijk} = random error term

All data were expressed as mean ± SEM. Differences were considered significant at the level of $P<0.05$.

Chapter IV

Results and Discussion

4.1. Conception rate

The conception rates observed in different experiments are shown in Figure 1. In *Experiment I*, the average conception rate was not significantly ($P>0.05$) higher in the does (70%) treated with $PGF_2\alpha$ (supplied by Techno Drugs Ltd. Bangladesh) than the does were under control group (65%). However, in *Experiment III,* treatment with $PGF_2\alpha$ supplied by Bayer (New Zealand) improved ($P<0.05$) the conception rate was higher (92.86%) compared to the does (control) treated with saline (85.71%). The receptivity and conception rate were higher ($P<0.05$) in the does (83.33%) treated with intra-vaginal washing with acetic acid than the does (72.22%) treated with normal saline (*Experiment II*). Among the infertile rabbit does, 50% conception was achieved by the treatment with $PGF_2\alpha$ and GnRH combindly (*Experiment III*). The present results are agreed with the other results. Dragan *et al*. (1996) suggested that conception rate is better from 85 to 95% of does treated with PG analogues compared to the controls. Hassanein (2000) found that the percentage of mating acceptance was 100% when administering 2.5 mg of Lutalyse® ($PGF_2\alpha$ analogue) before natural mating. Theau-Clement and Roustan (1992) found relation between receptivity and each of ovulation or fertilization. In their study, they found 100 and 88% receptivity and fertility rate after natural mating in receptive females and 13 and 10% receptivity and fertility in non-receptive females. $PGF_2\alpha$ or its analogues have been employed with the aim of synchronizing oestrous and improving the fertility rate of lactating does artificially inseminated (Boiti *et al*., 2006; Theau-Clement, 2008 and Gogol, 2009). Hormonal treatment may be attributed to the follicle stimulating effect of GnRH (Seleem, 2003), which improves receptivity through elevated estrogen levels. Our results revealed that the treatment with $PGF_2\alpha$ and GnRH combindly helped to overcome the infertility of rabbit does. Theau-Clément and Lebas (1994) also noticed increased estrus behavior in female rabbits after GnRH treatment and the rate of pregnancy increased approximately double of control. This result enables to say that the $PGF_2\alpha$ may be improved conception rate of does. Zapletal and Pavlik (2008) reported that the conception rates were improved from 10.0 to 89.5% by treatment with GnRH.

4.2. Gestation period

The gestation periods observed in different experiments are shown in Figure 2. In *Experiment I*, the average gestation periods were 30.67 and 30.14 days in control (T_0) and treatment (T_1) group, respectively. In *Experiment II*, gestation periods were 31.0 and 30.10 days in control (T_0) and treatment (T_1) group, respectively. In *Experiment III*, the average gestation periods were 30.40, 30.17 and 30.12 days in control does of fertile group (T_0), treated does of fertile group (T_1) and treated does of infertile group (T_1If), respectively. In all experiments, the gestation periods ranged from 30 to 32 days. However, there were no significant differences regarding gestation period among either treatment groups or control groups of the experiments. Gestation period in different breeds of rabbits ranged between 28-36 days (Morimoto, 2009). Rabbit does normally kindle 31-33 days after mating or artificial insemination (Rashwan *et al*., 2003). The average gestation lengths for Chinchilla, Dutch belted and New Zealand White rabbit were 30.50, 30.54 and 30.51 days, respectively (Addass *et al*., 2010). Tahir (2001) found that pure New Zealand White rabbit breed had longest gestation length (33 days). Inducing parturition of does by synthetic PG analogue (cloprostenol) injection intramuscularly on day 28 or 29 of pregnancy significantly reduced the length of pregnancy when compared with untreated does (McNitt *et al*., 1997 and Lavara *et al*., 2002). However, Dorra *et al*. (1997) reported that gestation period was approximately 31 days and was not affected by $PGF_2\alpha$ intrauterine injection.

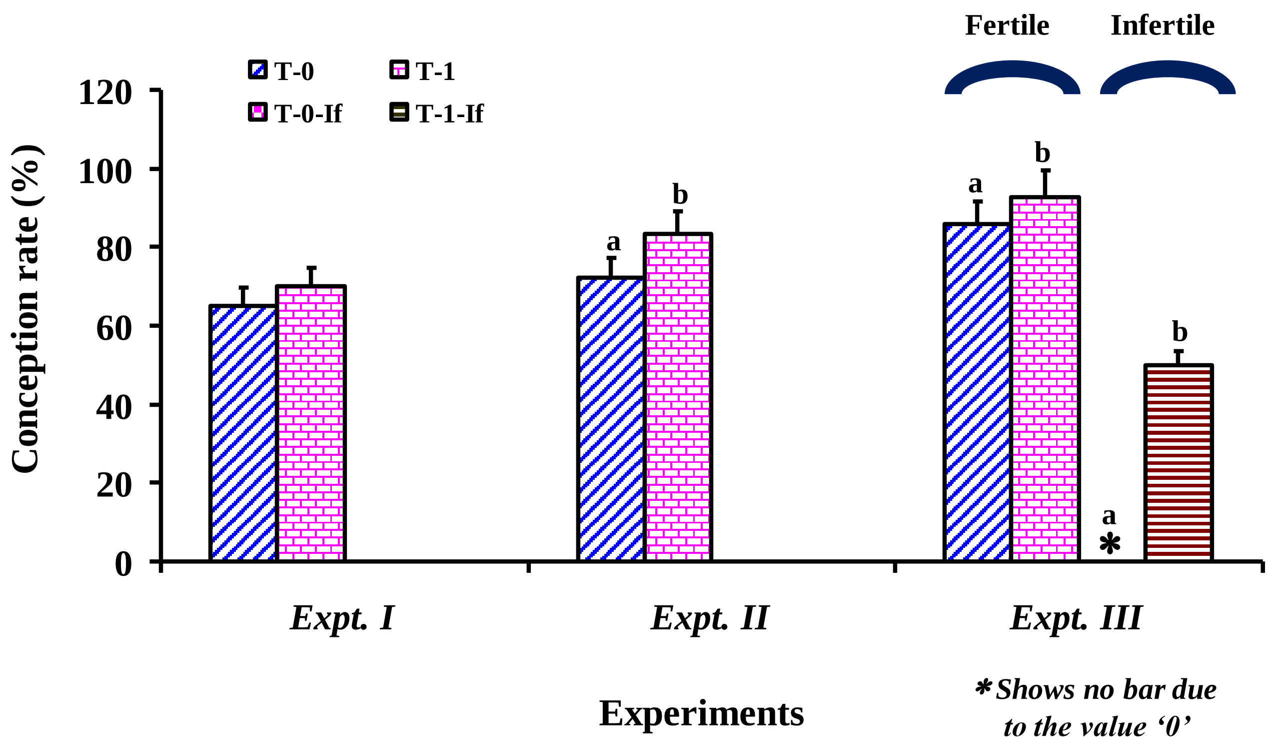

Figure 1. Conception rate of rabbit in different experiments (*Expt. I*, *Expt. II* and *Expt. III*). Each bar with error bar represents Mean ± SEM value. Different letter on error bars indicate significant differences (P<0.05) among the groups within the experiment.

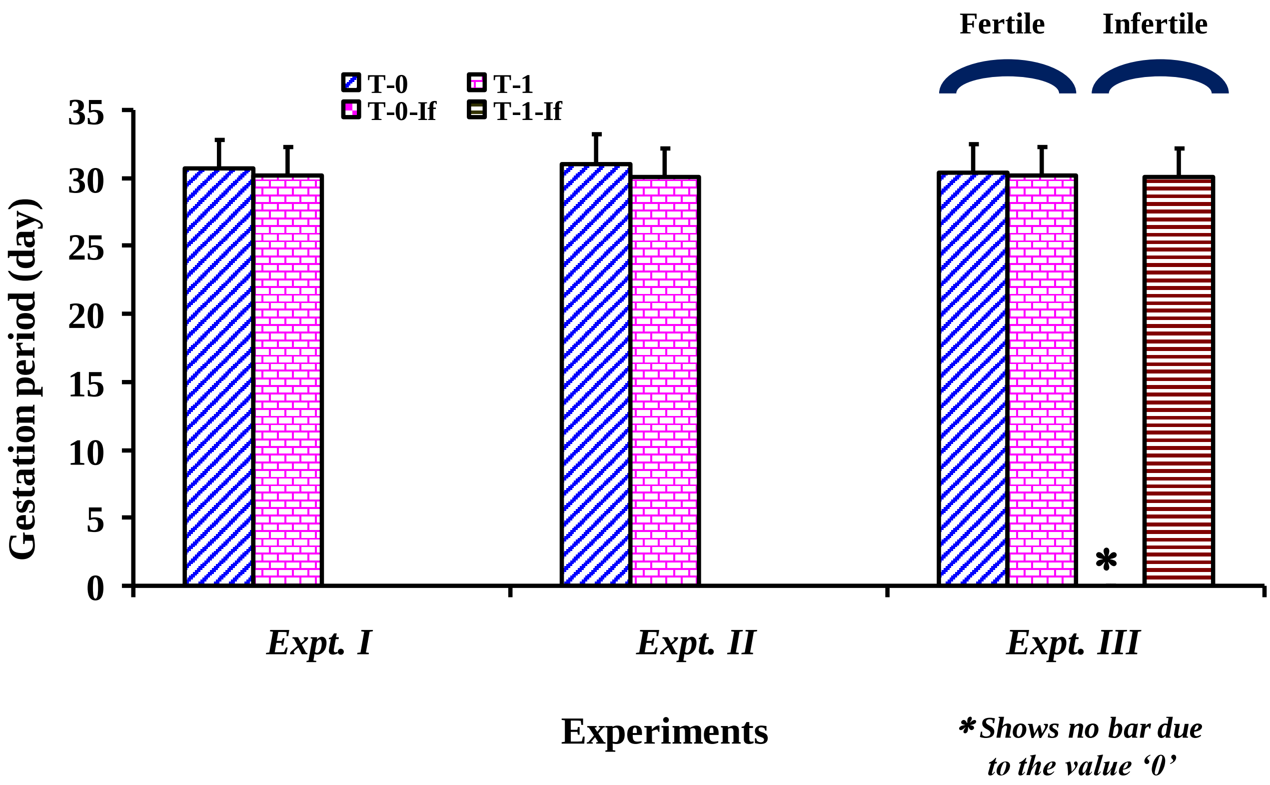

Figure 2. Gestation period of rabbit in different experiments (*Expt. I*, *Expt. II* and *Expt. III*). Each bar with error bar represents Mean ± SEM value. Differences were not significant (P>0.05) among the groups.

4.3. Litter size

4.3.1. Litter size at birth

The litter sizes at birth observed in different experiments are shown in Figure 3. In *Experiment III*, treatment with $PGF_2\alpha$ supplied by Bayer (New Zealand) significantly ($P<0.05$) increased the litter size at birth (4.31) compared to the does (control) treated with saline (3.42). But, in *Experiment I*, the average litter size at birth was not significantly ($P>0.05$) higher in the does (2.71) treated with $PGF_2\alpha$ (supplied by Techno Drugs Ltd. Bangladesh) than the does were under control group (2.69). The litter size was also higher ($P<0.05$) in the does which were treated with intra-vaginal washing of acetic acid (3.06) than the does (2.69) treated with normal saline (*Experiment II*). Among the infertile rabbit does, average litter size of 4.0 was obtained by the treatment with $PGF_2\alpha$ and GnRH (*Experiment III*). The present findings are in agreement with other findings. Fertility of rabbit does in terms of litter size at birth improved significantly by $PGF_2\alpha$ injection (Abd El-Glil, 1993; Kirrella *et al*., 1995; Dorra *et al*., 1997 and Gogol, 2009). Moreover, the intrauterine injection by $PGF_2\alpha$ may active the uterine contraction, which leads to the increase of sperm number and hence ova fertilization as reported by Kirrella *et al*. (1995) and Dorra *et al*. (1997). The litter size was 4.43 in control group compared to 8.46 in treatment group of V-line rabbit does (Abdel-Azeem, 2010). In the present studies, the overall litter size at birth were lower than the other studies, most probably due to the stress from hot-humid climatic condition of Bangladesh during the experimental period.

Abd El-Glil (1993) reported that when does mated within one hour or 48-72 hours after $PGF_2\alpha$ injection, the litter size at birth were improved and being 7.3 and 7.8 kits, respectively compared to the control does (5.6 kits). The number of kits kindled alive for Chinchilla, Dutch belted and New Zealand White rabbit was 4.92, 4.83 and 3.49, respectively (Addass *et al*., 2010). McNitt *et al*. (1997) indicated that total kits born per litter and kits born live per litter are the best by using $PGF_2\alpha$ analogues compared to control. On the other hand, Lavara *et al*. (2002) found that no significant differences in litter size at birth and live-born kits when used synthetic $PGF_2\alpha$ analogue (cloprostenol). Pimenta *et al*. (1996) obtained similar results (5.3 kits) with Mollo *et al*. (2003) after using natural $PGF_2\alpha$ in comparison to does treated with saline (4.4 kits).

4.3.2. Litter size at weaning

The average litter sizes at weaning observed in different experiments are shown in Figure 4. In *Experiment I*, no significant effect had been seen regarding the litter size at weaning (2.43 in the does treated with $PGF_2\alpha$ and 2.38 in the does treated with saline). However, in *Experiment III*, treatment with $PGF_2\alpha$ supplied by Bayer (New Zealand) increased ($P<0.05$) the litter size at weaning (4.08) compared to the does treated with saline (3.17). The litter sizes at weaning was not higher ($P<0.05$) in the does treated with intra-vaginal washing with acetic acid than the does treated with normal saline (*Experiment II*). An average litter size at weaning of 3.67 was achieved by the treatment with $PGF_2\alpha$ and GnRH, among the infertile rabbit does (*Experiment III*). Average litter size at weaning for Chinchilla, Dutch belted and New Zealand White rabbit were 2.78, 2.41 and 1.54, respectively (Addass *et al*., 2010).

4.4. Litter weight from birth to weaning

The litter weights from birth to weaning in different experiments are shown in Figure 5. In *Experiment II*, the litter weight was higher ($P<0.05$) in the does treated with intra-vaginal washing with acetic acid (163.6, 365.6, 528.13, 721.1 and 890.2 g litter weight, at birth, 7, 14, 21 and 28 days of age, respectively) than the does treated with normal saline (141.3, 322.4, 479.1, 641.2 and 795.5 g litter weight, at birth, 7, 14, 21 and 28 days of age, respectively). But, in *Experiment I*, the average litter weight at birth was not differed ($P>0.05$) by the use of $PGF_2\alpha$ hormone (supplied by Techno Drugs Ltd. Bangladesh) in comparison with the does treated with normal saline. The average litter weight at birth, 7, 14, 21 and 28 days of age were found 143.8, 301.9, 457.2, 590.5 and 751.1 g, respectively in the does treated with normal saline (T_0). Where, it was found 143.5, 295.6, 447.2, 589.3 and 734.8 g, respectively in the does treated with $PGF_2\alpha$ (T_1). However, in *Experiment III*, treatment with $PGF_2\alpha$ supplied by Bayer (New Zealand) increased ($P<0.05$) the litter weight (229.7, 470.0, 739.0, 946.1 and 1138.9 g litter weight, at birth, 7, 14, 21 and 28 days of age, respectively) compared to the does (T_0) treated with saline (182.7, 394.9, 605.2, 806.4, 992.7 g litter weight, at birth, 7, 14, 21 and 28 days of age, respectively). Among the infertile rabbit does, the litter weight (210.0, 442.4, 656.8, 836.0 and 1040.4 g litter weight, at birth, 7, 14, 21 and 28 days of age, respectively) was achieved by the treatment with $PGF_2\alpha$ and GnRH (*Experiment III*). The litter weight was positively affected by the litter size. The present results are in agreement with the other results. Addass *et al.* (2010) found similar results from the average litter weights at birth for Chinchilla, Dutch belted and New Zealand White rabbit (385.1, 436.8 and 317.7 g, respectively). But average litter weight at 28 days of age for Chinchilla, Dutch belted and New Zealand White rabbit (532.8, 504.0 and

334.9 g, respectively) were significantly lower (P>0.05) than the present findings (Addass *et al*., 2010). The litter weight at birth was higher (P<0.05) in treatment group (419.0 g) compared to control group (221.2 g) of V-line rabbit does (Abdel-Azeem, 2010). Abd El-Glil (1993) revealed that $PGF_2\alpha$ treatment affected the litter weight at birth and the average values were 395.9, 426.4 and 314.0 g in does received 100 µg $PGF_2\alpha$ and mated within one hour post-injection, or 48-72 hours post-injection and control does, respectively. The average litter weight at 21 and 28 days of age were lower in control group (1509.7 and 2232.4 g) compared to the treatment group (2087.2 and 3997.9 g) of V-line rabbit does (Abdel-Azeem, 2010), which are higher than the present findings.

4.5. Individual kit weight from birth to weaning

The individual kit weight from birth to weaning observed in different experiments is shown in Figure 6. In *Experiment I*, the average individual kit weight was not differed (P>0.05) by the use of $PGF_2\alpha$ hormone (supplied by Techno Drugs Ltd. Bangladesh) in comparison with the does treated with normal saline. The average individual kit weight at birth, 7, 14, 21 and 28 days of age were found 53.5, 112.2, 170.0, 219.5 and 279.2 g, respectively in the does treated with normal saline (T_0). Where, it was found 52.9, 109.1, 165.0, 217.4 and 271.1 g, respectively in the does treated with $PGF_2\alpha$ (T_1). In *Experiment II*, the individual kit weight was almost similar in the does treated with intra-vaginal washing with acetic acid (53.5, 119.5, 175.9, 235.6 and 290.9 g individual kit weight, at birth, 7, 14, 21 and 28 days of age, respectively) with the does treated with normal saline (52.5, 119.8, 178.1, 238.4 and 295.7 g individual kit weight, at birth, 7, 14, 21 and 28 days of age, respectively). However, in *Experiment III,* treatment with $PGF_2\alpha$ supplied by Bayer (New Zealand) decreased (P>0.05) the individual kit weight (171.47, 219.5 and 264.2 g at 14, 21 and 28 days of age, respectively) compared to the does (T_0) treated with saline (177.0, 235.8, 290.3 g at 14, 21 and 28 days of age, respectively). But individual kit weight at birth and 7 days of age were almost similar in does treated with $PGF_2\alpha$ (53.3, 109.1 g) and saline (53.4, 115.5 g). Individual kit weight among the infertile rabbit does was obtained (52.5, 110.6, 164.2, 209.0 and 260.1 g at birth, 7, 14, 21 and 28 days of age, respectively) by the treatment with $PGF_2\alpha$ and GnRH (*Experiment III*). The present results are agreed with the other results. Abdel-Azeem (2010) also observed that the individual kit weight decreased with the increased litter size among the $PGF_2\alpha$ treatment group of V-line rabbit does. He found that the individual kit weight, at birth, 21 and 28 days of age 52.5, 315.1 and 558.8 g, respectively in control group and 51.2, 264.7 and 538.6 g, respectively in treatment group of V-line rabbit does. Addass *et* *al*. (2010) found that the average individual kit weight, at birth, 7, 14, 21 and 28 days of age were 79.5, 108.4, 140.3, 163.8 and 186.1 g, respectively in Chinchilla, 90.8, 123.6, 160.3, 186.2 and 208.6 g, respectively in Dutch belted and 90.8, 120.9, 155.9, 186.4 and 218.4 g, respectively in New Zealand White rabbit. However, Lavara *et al*. (2002) observed that the birth weight of individual kit was significantly decreased in the does treated by $PGF_2\alpha$ compared to the control group (52 vs. 58 g).

4.6. Kit mortality

The kit mortality observed in different experiments is shown in Figure 7. In *Experiment III*, treatment with $PGF_2\alpha$ supplied by Bayer (New Zealand) decreased ($P<0.05$) the kit mortality (5.36%) compared to the does treated with saline (7.32%). But, in *Experiment I*, the average kit mortality was not different between the does (10.53%) treated with $PGF_2\alpha$ (supplied by Techno Drugs Ltd. Bangladesh) than the does were under control group (11.43%). However, the kit mortality was lower ($P<0.05$) in the does treated with intra-vaginal washing with acetic acid (8.70%) than the does (11.43%) treated with normal saline (*Experiment II*). Among the infertile rabbit does, kit mortality was 8.33% after the treatment with $PGF_2\alpha$ and GnRH (*Experiment III*). The present results are agreed with the other results. The kit mortality was 3.34% in control group compared to 5.59% in treatment group of V-line does rabbit (Abdel-Azeem, 2010). Hassanein (2000) found that the kit mortality was 12.1 and 27.7%, respectively in the does treated with $PGF_2\alpha$ and the does treated with saline. But, Abd El-Glil (1993) and Dorra *et al*. (1997) reported that there was no effect of $PGF_2\alpha$ treatment on viability. Addass *et al*., (2010) found 66% mortality for New Zealand White rabbit. However, McNitt *et al*. (1997) pointed out that does treated with $PGF_2\alpha$ analogue, cloprostenol and saline had intermediate numbers of live kits born.

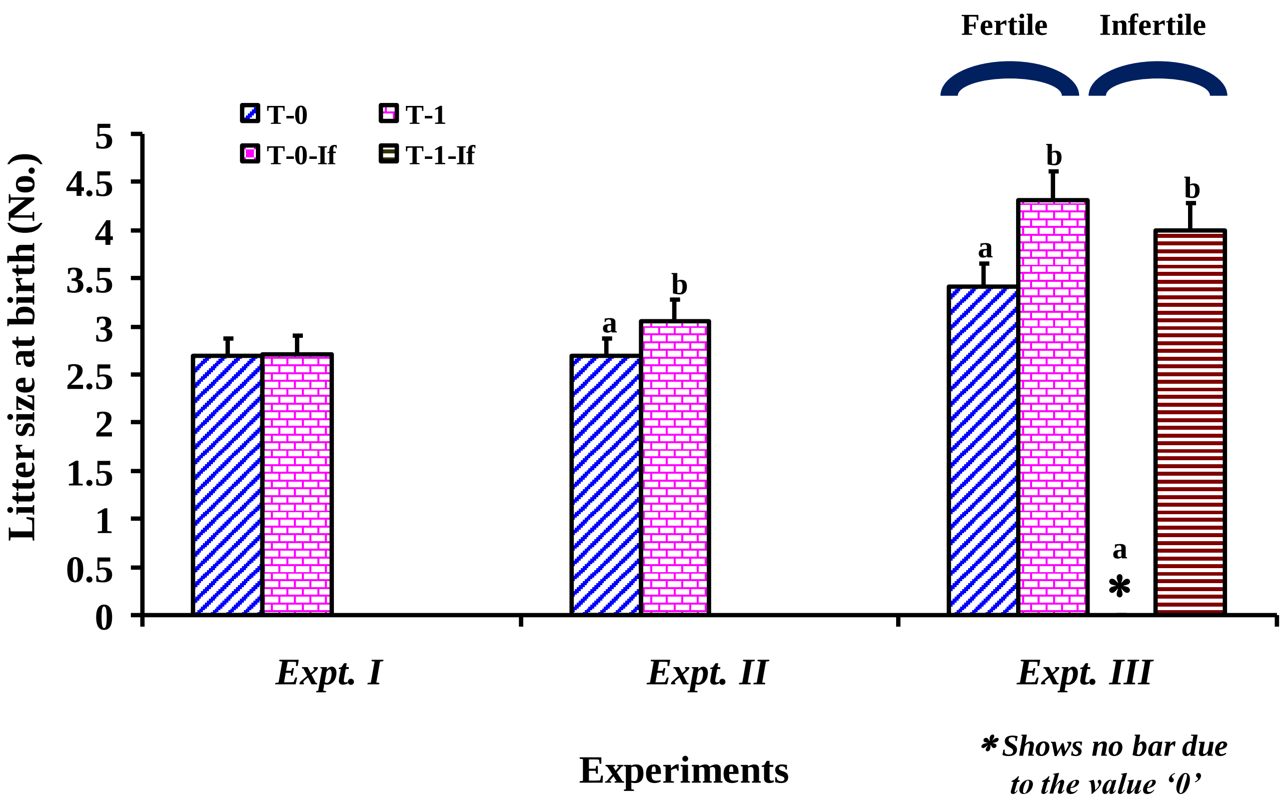

Figure 3. Litter size at birth in different experiments (*Expt. I*, *Expt. II* and *Expt. III*). Each bar with error bar represents Mean ± SEM value. Different letter on error bars indicate significant differences (P<0.05) among the groups within the experiment.

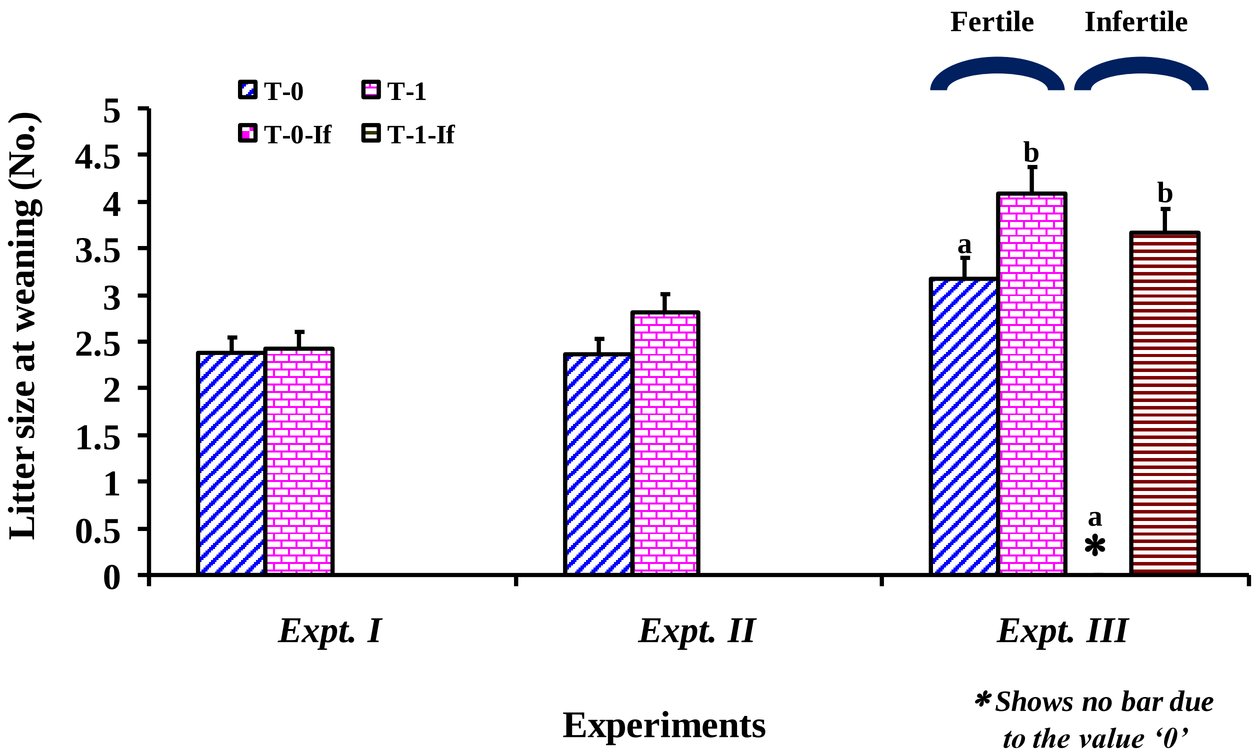

Figure 4. Litter size at weaning in different experiments (*Expt. I, Expt. II* and *Expt. III*). Each bar with error bar represents Mean ± SEM value. Different case letter on error bars indicate significant differences (P<0.05) among the groups within the experiment.

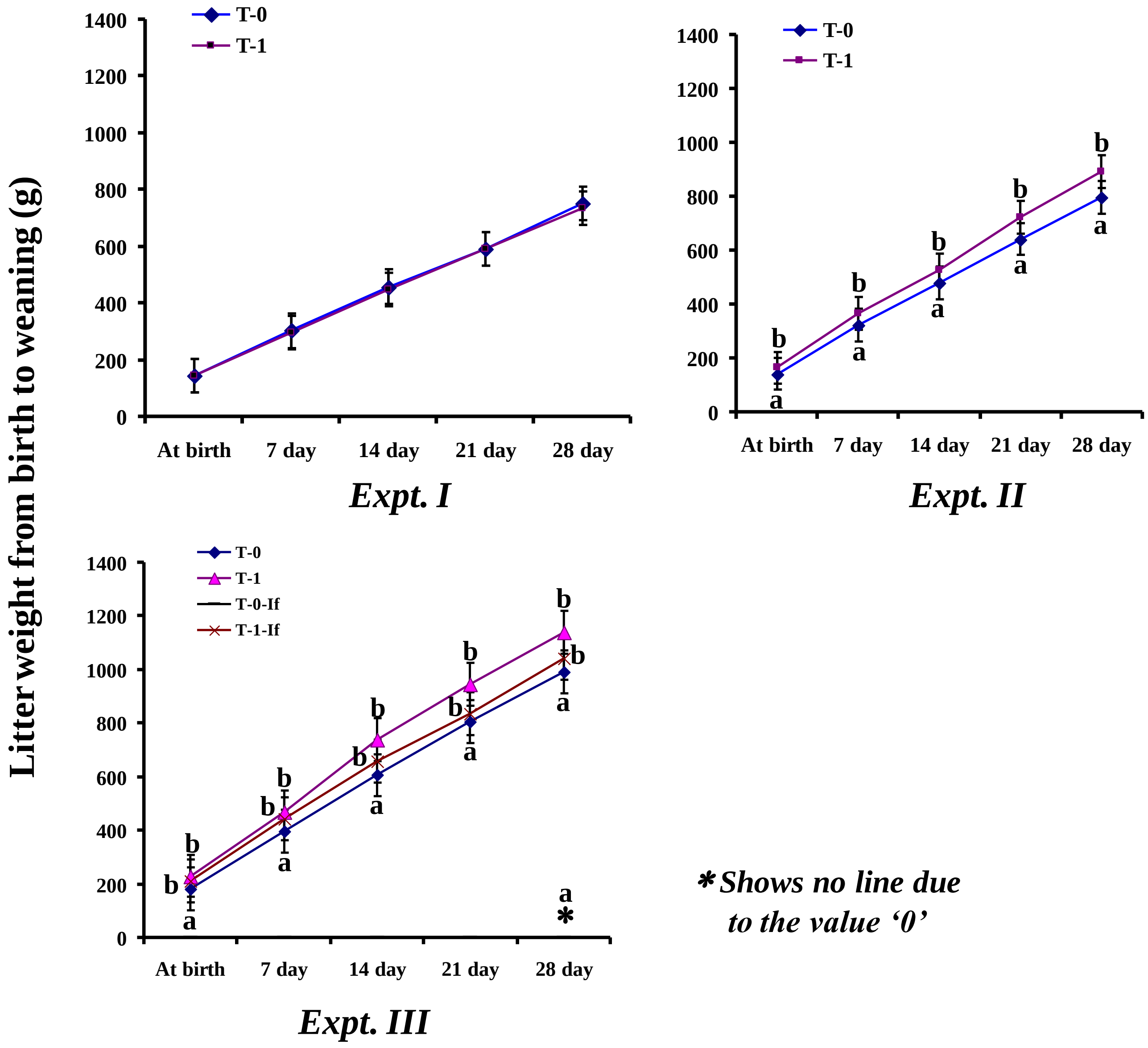

Figure 5. Litter weight from birth to weaning in different experiments (*Expt. I, Expt. II* and *Expt. III*). Each line with error bar represents Mean ± SEM value. Different letter on error bars indicate significant differences (P<0.05) among the groups within the experiment.

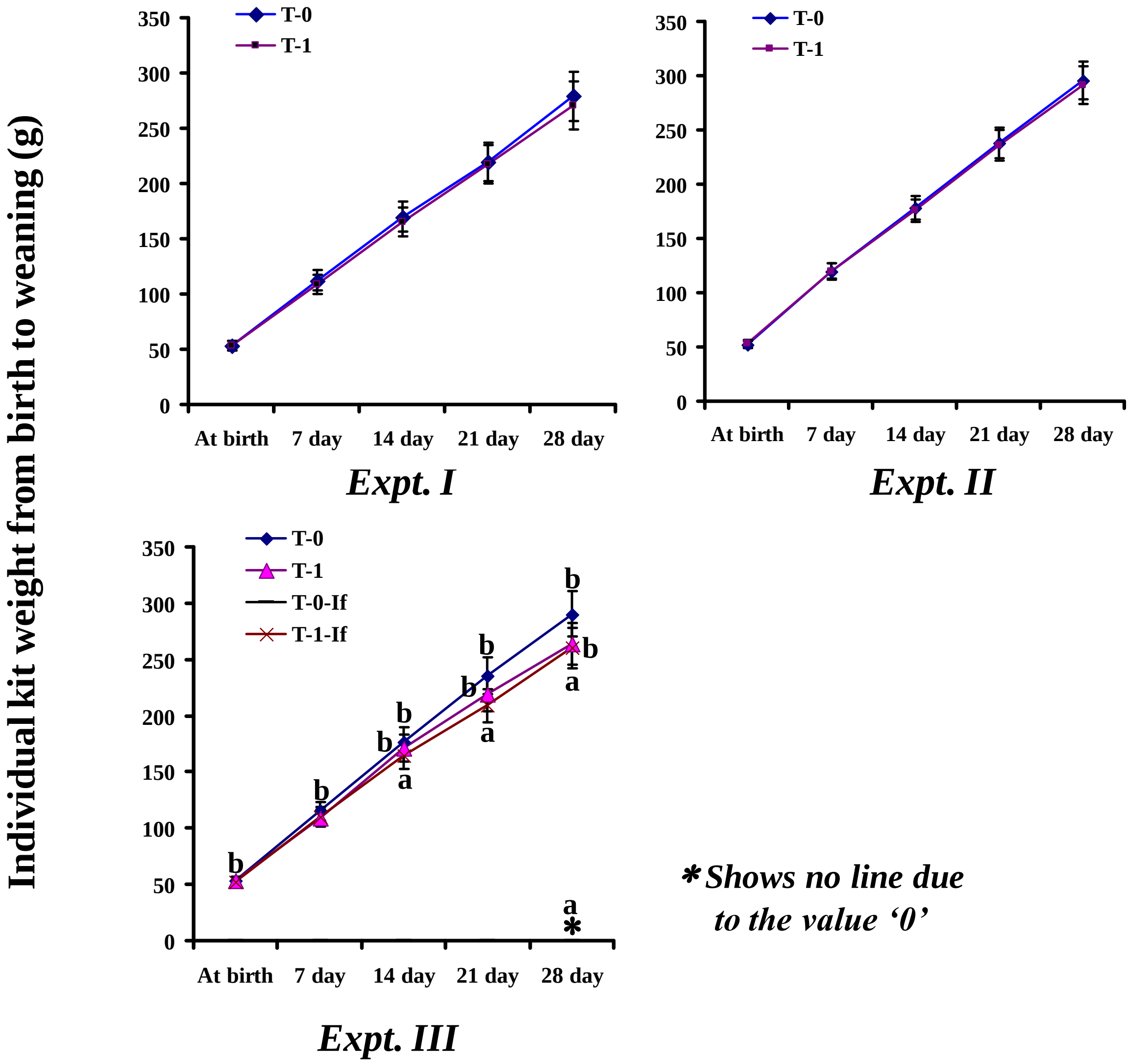

Figure 6. Individual kit weight from birth to weaning in different experiments (*Expt. I*, *Expt. II* and *Expt. III*). Each line with error bar represents Mean ± SEM value. Different letter on error bars indicate significant differences (P<0.05) among the groups within the experiment.

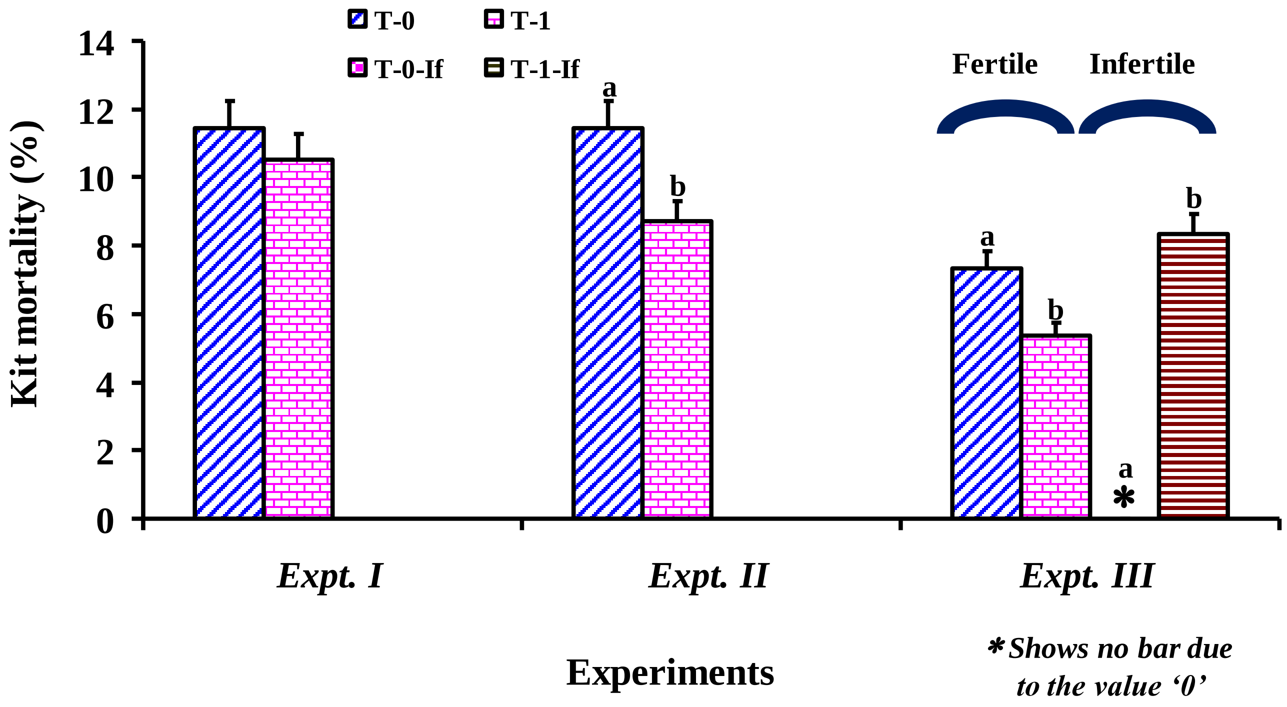

Figure 7. Kit mortality (%) in different experiments (*Expt. I*, *Expt. II* and *Expt. III*). Each bar with error bar represents Mean ± SEM value. Different letter on error bars indicate significant differences (P<0.05) among the groups within the experiment.

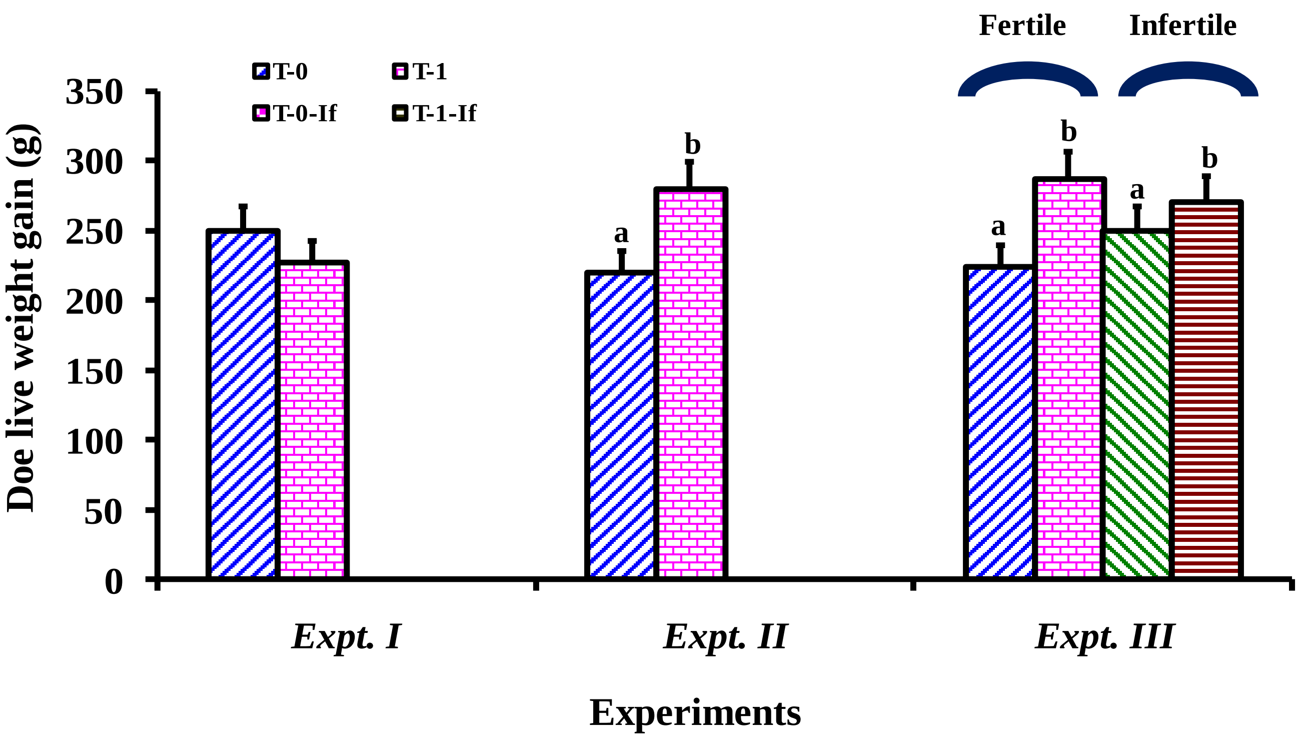

Figure 8. Doe live weight gain at 28 days of pregnancy in different experiments (*Expt. I, Expt. II* and *Expt. III*). Each bar with error bar represents Mean ± SEM value. Different letter on error bars indicate significant differences (P<0.05) among the groups within the experiment.

4.7. Live weight gain of rabbit does

The average live weight gain of does observed in different experiments is shown in Figure 8. In *Experiment I*, the average live weight gain of does were not significantly differed between the does (227.14 g) treated with $PGF_2\alpha$ (supplied by Techno Drugs Ltd. Bangladesh) and the does were under control group (250.0 g). However, in *Experiment III*, treatment with $PGF_2\alpha$ supplied by Bayer (New Zealand) significantly increased the live weight gain of does (286.6 g) compared to the does (224.0 g) treated with saline. The doe live weight gain was higher ($P<0.05$) in the does treated with intra-vaginal washing with acetic acid (280.0 g) than the does (220.0 g) treated with normal saline (*Experiment II*). Among the infertile rabbit does, the doe live weight gain (270.0 g) achieved by the treatment with $PGF_2\alpha$ and GnRH was not significantly higher than does (250.2 g) treated with normal saline (*Experiment III*). The present findings are in agreement with other findings. The live weight gain of does during pregnancy were 640.2 and 553.3 g in control and treatment groups of rabbits (Abeer *et al*., 2011). Dragan *et al*. (1996) found that the live weight gain of does during pregnancy were 402 and 359 g in two groups of chinchilla rabbits. While, Maertens *et al*. (1995) observed that, the live weight gain of does during pregnancy was 306 g in New Zealand White rabbits which is similar to the current findings.

4.8. Post mortem examination of different organs of rabbit

After performing three consecutive examinations, there were few rabbit does, which were not conceived for a single time. To investigate the reasons of infertility, post mortem examination were done with the reproductive and other organs of some low fertile and infertile rabbit does. In case of infertile rabbit does, the abdominal fat content (126.3 g) was higher ($P<0.05$) than fertile does (58.4 g). But other parameters such as heart weight (4.1 and 4.0 g in infertile and fertile does, respectively), blood volume (54.3 and 57.7 ml in infertile and fertile does, respectively), liver weight (63.2 and 58.7 g in infertile and fertile does, respectively), kidney weight (10.03 and 10.43 g in infertile and fertile does, respectively), vagina size (7.3 and 7.4 cm in infertile and fertile does, respectively), uterus size (8.3 and 8.4 cm in infertile and fertile does, respectively), oviduct size (4.53 and 4.44 cm in infertile and fertile does, respectively) and ovary size (1.3 and 1.4 cm in infertile and fertile does, respectively) were almost similar. Due to lack of minute pathological and histological investigation, the exact cause could not be found. Further investigation is required to find the specific cause of infertility in the rabbit does.

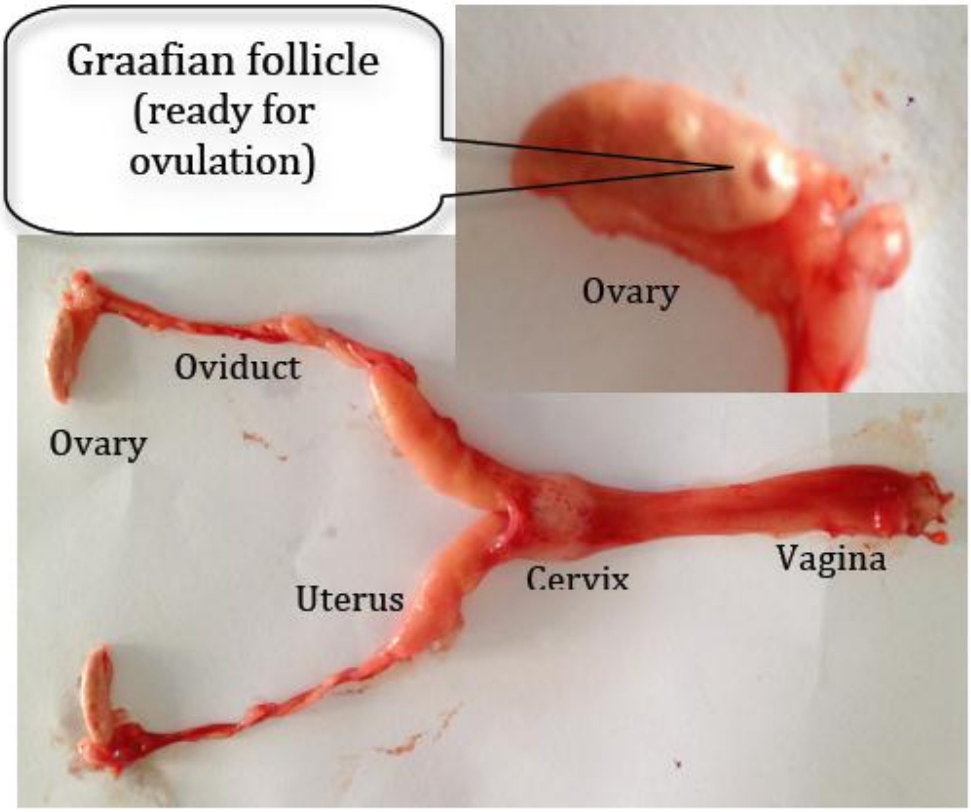

Photo 17. Reproductive organs of rabbit doe

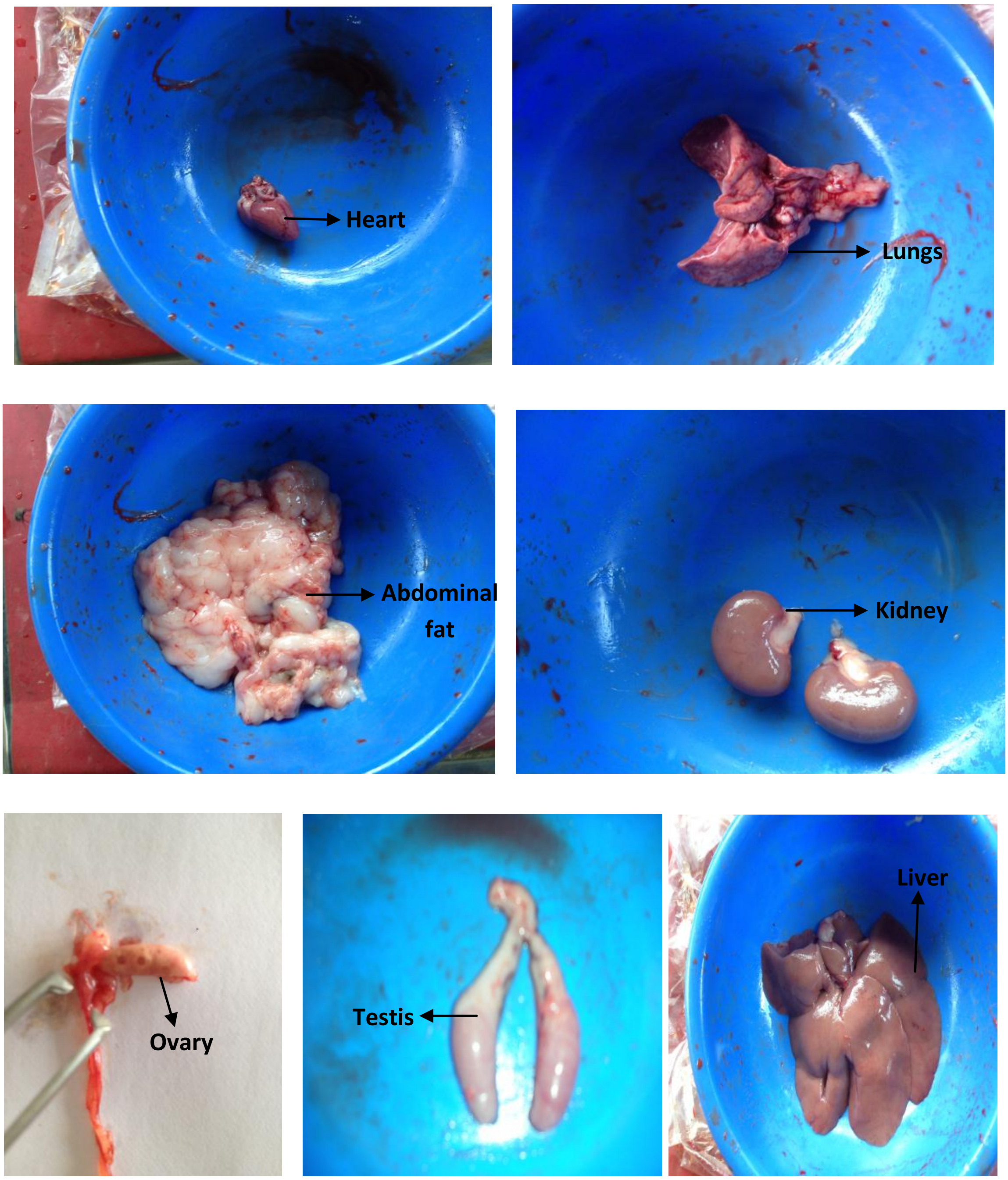

Photo 18. Heart, lungs**,** abdominal fat, kidneys, ovary, testis and liver of rabbit

Chapter V

Summary and Conclusion

In Bangladesh, due to the severity of poverty most of the people suffer from an acute shortage of animal protein. The poultry industry was the major source of animal protein, which has been destroyed due to the severity of avian influenza. Therefore, to mitigate the protein shortage, small-scale rabbit production may be an alternative protein source. But, major limitation for the development of small-scale rabbit production is the heat stress, which adversly affects productive and reproductive performance of rabbits. However, there is no study tailored towards the needs to over come the adverse effects of heat stress in small-scale rabbit production in Bangladesh. Therefore, the aim of this study was to investigate whether productive and reproductive performance of rabbit can be improved through physiological manipulation by conducting three on-station experiments (*Experiment I* was conducted from March to April 2014, *Experiment II* was conducted from May to July 2014 and *Experiment III* was conducted from August to September 2014.). In *Experiment I,* 20 rabbit does were treated with the hormones (0.25 m1/doe $PGF_2\alpha$), in *Experiment II,* 18 rabbit does were washed intra-vaginally with acetic acid (1 ml/doe) before mating and in *Experiment III*, 20 rabbit does were treated with hormones (0.25 m1/doe $PGF_2\alpha$ and 0.20 ml/doe GnRH) before mating. The productive and reproductive performances of rabbit does were recorded throughout the experimental periods and analyzed the observed data statistically. The present study suggests that hormones ($PGF_2\alpha$ and GnRH) and intra-vaginal washing with 1% acetic acid can be used for improving reproductive performance of rabbits does. Intra-vaginally washing with acetic acid could be effective in removing or decreasing the uterine infections which in turn reduce the life span of corpora lutea and increase mating acceptance.

The results might contribute to overcome the adverse effects of heat stress and improve productive and reproductive performances of rabbits in hot-humid climatic condition, as well as may open the door for the alternative protein source and may act as a weapon for poverty elevation in Bangladesh through small-scale rabbit production.

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Appendix: Rabbit Breeding Record

Rabbit No. (Doe): ------------------------ Breed: ------------------------- Source: ------------------------ DOB: ---------------------------- Sire: ---------------

Note: ---

Sl. No.	WBB	BD	BN	PD	ND	WBN	KD	WAK	LSB	SB	LWB	IKWB	LW 7D	IKW 7D	LW 14D	IKW 14D	LW 21D	IKW 21D	LW 28D	IKW 28D	LSW	Remarks

Where, DOB- Date of birth, WBB- Weight before breeding, BD- Breeding date, BN- Buck number, PD- Pregnancy diagnosis, ND- Nest giving date, WBN- Weight before giving nest, KD- Kindling date, WAK- Weight after kindling, LSB- Litter size at birth, SB- Still birth, LWB- Litter weight at birth, IKWB- Individual kit weight at birth, LW 7D- Litter weight at 7 day, IKW 7D- Individual kit weight at 7 day, LW 14D- Litter weight at 14 day, IKW 14D- Individual kit weight at 14 day, LW 21D- Litter weight at 21 day, IKW 21D- Individual kit weight at 21 day, LW 28D- Litter weight at 28 day, IKW 28D- Individual kit weight at 28 day and LSW- Litter size at weaning.