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IMPACT OF HUMAN MENOPAUSAL GONADOTHROPHIN (PERGONAL)$^{(R)}$ ON SEMEN CHARACTERISTICS OF MATURED MALE RABBITS

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Abstract

Twenty four (24) sexually matured male rabbits between the ages of 5 – 8 months were used to evaluate the effect of Human Menopausal Gonadotrophin (HMG) on the semen characteristic of the matured rabbits bucks. The twenty four male rabbits were divided into four treatment groups identified as: R_1M (which served as the control group) R_2M, R_3M and R_4M. Rabbit bucks in R_1M (control) were administered with 0.00ml of HMG, R_2M group received 0.03ml of HMG, R_3M also received 0.06ml of HMG, while group R_4M was administered with 0.09ml of HMG injection. The animals were randomly assigned into four treatments of HMG in a completely randomized design (CRD). Each group was further divided three times, giving two animals per replicate. HMG was administered to the animals for three consecutive days. Semen collection from the male reproductive organ of the rabbits was done after five days of administration of the drug. The semen was collected using matured cycling doe to tease the buck making use of an artificial vagina (AV). The semen colour (creamy milky) obtained in this study was similar in all treatment groups. Semen volumes, semen pH, sperm concentration of the semen, live sperm cells values differed significantly between treatment groups and control group. Higher values on semen characteristics were obtained in R_4M group. On the other hand the percentage dead sperm cells, sperm motility, and libido levels were significantly ($p<0.05$) higher in the control group than those in the other treatment groups. This shows that the HMG had a positive influence on the semen characteristics of rabbit bucks.

Keywords: Impact, Human Menopausal Gonadotrophin, (Pergonal$^{(r)}$), Semen Characteristics, Male, Rabbits

Introduction

The domestic rabbit is believed to have originated from wild rabbits found in the Mediterranean countries (Clutton, 1999 and Sussan *et al,* 2003). These rabbits were distributed to various parts of the world by salors who wish to have readily available source of meat at various points on their voyages. This must be the most likely means of early introduction of rabbits in Nigeria (Aduku and Olukosi, 1990).

Rabbits are among the micro livestock reared by man. They are small in size and can be reared by both children and women. They weigh about 2 – 3kg and attains to maturity at the age of 3 – 4 months. Domestic rabbits provide monetary income to their owners quickly (Sherman, 2002) as they have fast growth and quick reproduction rates. Rabbits play an important role in research work among students and teachers (Stifel, 1990) and responds well to intensive management system unlike animals such as sheep, goat and cattle. This makes it easy for small holder farmers to be able to produce them (Ajakemo, 2014). All these have led to the growing interest in rabbit production in the third world and caused it to be incorporated into the current livestock aid in Nigeria (Herbert, 1992 and Alvarino, 2000).

All these led to the study of semen characteristics of male rabbits administered with Human Menopausal Gonadotrophin (HMG) also known as pergonal(r).

Human menopausal gonadotrophin (HMG) or personal(r) is a fertility drug that consists of 1:1 ratio of follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Dixon and Hopkins, 1996). These hormones play vital roles in the initiation of spermatogenesis in the male animals (Herbert *et. al.,* 2000 and Abu *et. al.,* 2006). In females, the luteinizing hormone plays an important role in the induction of ovulation. Looking at these, this study therefore deemed it necessary to look at the effect of HMG on semen characteristics of male rabbits.

Materials and Methods

This study on semen characteristics of rabbits administered with Human Menopausal Gonadotrophin (pergonal(r)) was conducted at Abia State University teaching farm at Umudike – Umuahia. Abia State University, (Umuahia campus). It is a place characterized with luxuriant grasses and legumes that are quite ideal for rabbit feeding and sandy loam soils with pH range of 6.0 – 7.0 (Adiele *et. al.,* 2005). This area is characterized with mean ambient temperature of about 35^0C.

Experimental Animals:

Healthy rabbits were sourced from local farms and research institutes around the experimental site. Twenty four (24) sexually matured male rabbits comprising of twenty four bucks aged between 5 – 8 months were used to evaluate the semen characteristics of mature bucks administered with human menopausal gonadotrophin (HMG). These animals were given 2 weeks pre experimental periods to get themselves acclimatized to the environment and to the experimental procedures as well. Each animal was kept in a hutch measuring 50 x 50cm and each hutch was labeled for easy identification of the rabbits used for the experiment. Routine inspections were carried out on daily basis. The animals were dewormed two times within the experimental periods. The actual experimental periods lasted for 12 weeks.

Experimental Diets and Composition:

The experimental rabbits were maintained on a freshly cut forages made up of grasses and legumes such as *pennisetum purpureum, centrocema pubescence* and *pannicum maximum*. Through chemical analysis, the proximate composition of these plants was determined. The proximate analysis fractions determined includes the following – dry mater content, moisture content, crude protein, either extract, nitrogen free extract, crude fibre, ash content in accordance with AOAC (1990).

Experimental Design

Twenty four matured rabbits' bucks that were used for this study were randomly assigned to four treatment groups identified as R_1M, R_2M, R_3M, and R_4M. In a completely randomized design (CRD). Each group was further, sub-divided into 3 replicates of 2 mature rabbit bucks. Each treatment group was housed in a separate cage, where they received different doses or level of pergonal$^{(r)}$. Group R_1M served as the control group and did not receive any pergonal$^{(r)}$ treatment. Group R_2M was administered with 0.03ml of pergonal daily for 3 consecutive day. Group R_3M and R_4M received 0.06ml and 0.09ml pergonal$^{(r)}$ treatment respectively for 3 consecutive days.

The fertility drug was administered to each male rabbit bucks intramuscularly at the buttocks near the hindlegs using insulin syring with 0.1 graduation. After the fifth day of the administration of the drugs, semen was collected from the bucks for semen analysis. Reaction time was also observed and recorded. A matured cycling doe (teaser) was introduced to the buck every week, in order to monitor their sex drive. In this study, reaction time was considered as an indicator of libido, that is the time in seconds it took the buck to sniff, grunt and mount the female. All these were recorded using a stop watch and libido was scored using the scoring pattern described by Chibundu (2005).

Collection and Characterization of Semen

Semen collection was usually done before 8.00am and 10.00am during the breeding period of the experiment in order to ensure that quality semen was obtained. A matured cycling doe was always used to tease the buck which made it to thrust in an attempt at intromission. At this point, a pre-warmed and treat artificial vagina (AV) was carefully introduced from the side, making it to be in contact with the erect penis of the buck, which makes deep thrust and ejaculation to take place in a matter of seconds. The semen colour collected was dictated by visual appraisal with the use of a transparent test tube. The volume of semen collected was measured using graduated collection tube. The semen pH was determined with raw semen using a 507 crison pH meter. Semen concentration per ejaculate was calculated as: Sperm concentration per ml x volume per ejaculated, (Egbuka, 1995).

Sperm concentration per ml was evaluated using a visual count under the light projecting microscope at x 10 magnification using Neubauer hamocytometer, after the sperm cells have been immobilized with 1% formaldehyde solution.

The percentage live and dead sperm cells were determined by using a microscope to observe a mixture of a drop of semen and a drop of eosin-nigrosin dye on a slide. The percentage of the live and dead spermatozoa was assessed by identifying those with an intact cell membrane from dye exclusion. The spermatozoa that absorb stains are said to be dead, while those that did not absorb the stain are not dead (live sperms).

The sperm progressive motility was obtained by a diluting drop of semen with a saline (0.9% NaCl) at the same temperature. A sterile rod was then used to place a drop of the diluted semen on a warm slide. The sperm motility was observed under the microscope at (x10) and (x40) magnification and scored objectively using the scoring pattern described by Omalako (1992).

Results

The results of the study on the impact or effects of Human Menopausal gonadotrophin on semen characteristics of matured rabbit bucks are presented in table I of this study. Data obtained on semen volume were within the range of 0.58 – 0.64m. The male rabbits in treatment group R_4M had the highest value in semen volume (0.64ml) which is higher than those in the control group, (R_1M), R_2M and R_3M treatment groups, with 0.58ml, 0.59ml and 0.60ml respectively.

Table 1: Effect of Pergonal$^{(r)}$ on Semen Characteristics of Matured Male Rabbit

Parameters	Treatment				Sem
	R_1M	R_2M	R_3M	R_4M	
Semen colour	Cream milky	Cream milky	Cream milky	Cream milky	
Semen volume (ml)	0.58^{d}	0.58^{c}	0.60^{b}	0.64^{a}	0.01
Semen pH	8.11^{d}	8.15^{c}	8.20^{b}	8.22^{a}	0.04
Sperm concentration ($x10^{9}$/ml	0.84^{d}	0.86^{c}	0.88^{b}	0.89^{a}	0.10
Live sperm (%)	72.10^{d}	84.20^{c}	86.10^{b}	88.20^{a}	3.61
Dead sperm (%)	28.10^{a}	15.34^{b}	14.41^{c}	12.20^{d}	4.29
Sperm motility (%)	68.48^{a}	60.12^{b}	55.69^{c}	53.34^{d}	3.34
Libido (reaction time)	4.20^{a}	4.15^{b}	4.05^{c}	4.03^{d}	0.03

a,b,c,d: means within rows having different superscripts are significantly different ($p< 0.05$)

R_1M received 0.00ml pergonal$^{(r)}$

R_2M received 0.03ml pergonal$^{(r)}$

R_3M received 0.06ml pergonal(r)

R_4M received 0.09ml pergonal(r)

Discussion

One of the methods of assessing reproductive efficiency of the male animals is through the measurement of semen quality. In this study variations did not exist in semen colour of bucks administered with pergonal(r) from those in the control group, indicating that the drug pergonal(r) did not effect any change on the colour of the semen. According to Iheukwumere *et. al.,* (2008), the predominant colours of semen are creamy white and light yellow. Any colour variation of semen may be as a result of contamination with feaces, urine and blood (Ameh, 2004). In this study, semen colours observed among the treatment groups were coloured creamy milky only. This indicated that semen was not contaminated during semen collection. Secondly the drug pergonal(r) did not alter the normal colour of the semen of the rabbit bucks. These findings agree with the work of Iheukwumere (2008), that the appearance of semen is a part of important characteristics of semen quality.

The semen volume – 0.58 – 0.64ml obtained in this study showed that the administration of different doses of pergonal at 0.03ml, 0.06ml and 0.09ml levels caused an increase in semen volume values and this result goes in line with the findings of Castellini (2008) and Nizza, Meo and Taranto (2000) who observed higher semen volume in semen characteristics of rabbit bucks.

The semen pH value obtained in this study (8.11 – 8.22) fell within the normal range of 8.04 – 8.35 reported by Baiti *et. al.,* (2005) and Ogbuewu et. al., (2009), but below the range reported by Iheukwumere and Okereke (1990) in yankasa ram. The highest pH value recoded in group R_4M (8.22), showed that the drug pergonal(r) had significant effect on the pH value of the male rabbits. This however agreed with the findings of Ogbuewu *et. al.,* (2009).

The sperm concentration obtained in this study (0.84 – 0.89 x 10^9/ml) fell below the range (2.01 – 2.08 x 10^9/ml) reported by Iheukwumere *et. al.,* (2008) and Castellini (2008). The observed differences may be differences in the levels of pergonal administered to the animals. Actually, the rabbits in R_4M recorded the highest value in sperm concentration (0.89 x 10^9/ml), showed that the drug pergonal(r) had significant effect on the sperm concentration of the rabbit.

The values of the average percentage live sperms obtained in treatment groups R_2M – R_4M (84.20 – 88.20%) fell above the range (51.12 – 54.70%) reported by Ariotta *et. al.,* (2000) and Ameh *et. al.,* (2004). High live sperm cell counts have been found to be vital in fertility of

animals (Johnson, 2004). This however, showed that the drug pergonal(r) had a significant effect on the live sperm percentage of the treated male rabbits used for this study.

The mean percentage of dead sperm cells obtained in this study, which ranged from 12.20 – 28.10% fell within the range of 12.18 – 28.3% reported by Iheukwumere *et. al.,* (2008) but was lover then 21.0 – 22.5% reported by Abu *et. al.,* (2006). The result obtained in this study however showed that the drug pergonal did not have harmful effect on the sperm cells of the animals, as the percentage values of dead sperm cells maintained a downward trend as the levels of pergonal injection increased.

This study also revealed that there was a progressive decline in percentage of motile sperm cells in ejaculates of bucks in R_2M, R_3M and R_4M treatment groups compared to those in the control group (R_1M). However, the percentage motility values obtained from groups R_3M to R_4M (60.12 – 53.34%) fell within the range of 48.3 – 57% reported by Moce *et. al.,* (2005), while the values obtained in R_1M and R_2M fell within the range of 60.01 – 69.20% reported by Iheukwumere *et. al.,* (2008). The differences observed in sperm motility may be as a result of breed (Oguike, 2000) and hormonal effect of the drug which must have affected the sperm cells weight and motility rate as well. Sperm motility is an important index in reproduction assessment because it demonstrates the ability of the sperm to move and fertilize an ovum.

Libido was found to be higher in rabbits in treatment groups R_1M and R_2M (4.20 and 4.15) respectively and lower in bucks in treatment groups R_3M and R_4M (4.05 and 4.03). The lower Libido observed in groups R_3M and R_4M fell below the range 4.06 to 4.15 reported by Iheukwumere and Okereke (1990) in Yankasa ram, but fell within the range reported by Ndubueze (2002). The variation could be attributed to the effect of the drug on the buck that caused the animals to store high volume of semen which made ejaculates to take place so quickly immediately the does were introduced to the bucks in R_3M and R_4M.

Conclusion

The results obtained on the semen characteristics of the bucks administered with pergonal(r) injection at different levels showed that the drug did not effect any change in colour of the semen in all the treatment groups. The drug also caused an increase in the semen characteristics values obtained in this study, except in sperm motility and sexual libido values where the rabbits in the control groups scored higher than those in the treatment groups R_2M to R_4M.

Recommendation

As regards to the results obtained in this study, it is therefore recommended that 0.09ml of pergonal should be administered to rabbits and other male animals in order to improve their semen quality. Oncemore extra work should be carried out on the use of HMG as a fertility enhance for animals as it was observed from literature that, only few author have done some work on the use of pergonal as a fertility enhancer in domestic animals.

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