Banat's Journal of Biotechnology
Contact: web: http://www.bjbabe.ro, e-mail: contact@bjbabe.ro

EFFECT OF GONADOTROPHIN (DICLAIR®) ON SEMEN CHARACTERISTICS, HORMONAL PROFILE AND BIOCHEMICAL CONSTITUENTS OF THE SEMINAL PLASMA OF MATURE BALAMI RAMS

DOI: 10.7904/2068–4738–VIII(15)–90

Uche Ndubuisi EGU[1] Josiah Chidiebere OKONKWO[2]

[1]*Department of Animal Science and Fisheries, Abia State University, PMB 7010, Umuahia, **NIGERIA***
[2]*Department of Animal Science and Technology, Nnamdi Azikiwe University, PMB 5025 Akwa, **NIGERIA***
Corresponding author: ucheegu1@gmail.com, jc.okonkwo@unizik.edu.ng

Abstract. Twelve sexually matured (2–2.6 years old) healthy Balami rams were used to determine the effect of Gonadotrophin (Diclair®) on semen characteristics, body conformation and hormonal profile. The rams were divided into 3 treatment groups of 4 rams, identified as T_1 (control), administered with 1.00 mL physiological saline, T_2, administered with 54.00 i.u Diclair® (0.36 mL), T_3, administered with 108.00i.u Diclair® (0.72 mL) with one ram per replicate in a Completely Randomized Design (CRD). The injections were divided into three doses each and administered intramuscularly in the thigh for three consecutive days. The results showed that there were significant differences ($P < 0.05$) among the treatment groups in all the parameters for semen quality: semen volume, individual motility, sperm concentration, proportions of live, normal and dead sperm cells except semen pH which was similar ($P > 0.05$) among the treatment groups. The results further showed that there were significant differences ($P < 0.05$) among the treatment groups in luteinizing hormone (LH) and testosterone levels in the serum. However, there were no significant differences ($P>0.05$) among the treatment groups follicle stimulating hormone(FSH) level in the serum. Similarly, the results showed that there were significant differences ($P < 0.05$) among the treatment groups in urea, glycerophosphocholine, ascorbic acid, sodium, potassium, bicarbonate and fructose levels in the seminal plasma. The results of this study showed that Diclair® enhanced semen quality and was not detrimental to the hormonal profile and biochemical constituents of the seminal plasma of the Balami rams.
Keyword: Diclair®, semen quality, hormones, seminal plasma constituents, Balami rams.

Introduction

The Balami sheep is the biggest of the Nigerian sheep breed and is found mainly in the drier Sudan and Sahel Savannah zones.

The Balami sheep is the biggest of the Nigerian sheep breed and is found mainly in the drier Sudan and Sahel Savannah zones.

It has an all-white coat, convex face with large and droopy ears and long tail.

Males have horns while females are hornless. Matured males (ram) may have dewlap and weigh between 40–65 kg. Females weigh 30–45kg.

The Balami sheep is also referred to as dessert breed. It is a good dairy breed [ONI, 2002].

The primary aim of induction of spermatogenesis is to improve semen quality [AMEH, 2004; ABUT *et al.*, 2006].

Spermatogenesis involves the use of follicle stimulating hormone (FSH) and luteinizing hormone (LH) [ABU *et al.*, 2006].

Most of these preparations of FSH and LH are very expensive perhaps because of the brand names; some of them require cold chain storage and often deteriorate because of inadequate storage and handling [HERBERT *et al.*, 2000].

There is therefore the need to examine some generic preparations that could induce spermatogenesis in the animals but at the same time are cheap, readily available and easily managed under developing countries conditions.

Diclair®, a fertility drug also known as Humagon or mentrophin is a gonadotrophin preparation lyophilized in vials containing a mixture of gonadotrophins consisting of FSH (DFSH) and LH (DLH) in a ratio 1:1 (Dixon and

Available on-line at http://www.bjbabe.ro
Banat's Journal of Biotechnology
2017, VIII(15)

Hpkins, 1996). Follicle stimulating hormone and LH present in Diclair® play vital role in the initiation of spermatogenesis [ABU *et al.*, 2006].

It has not been determined if the administration of the hormone preparation for spermatogenesis would induce any side effects on the hormonal profile and seminal plasma constituents of the Balami rams.

This study was therefore carried out to determine the effects of Diclair® administration on the semen quality, hormonal profile and seminal plasma constituents of Balami rams.

Material and methods

Experiment Animals and their Management

Twelve healthy, sexually matured Balami rams aged 2–3 years were used for this study. The animals were purchased from the local markets and housed in clean pens constructed in such a way that the rams could come outside during the day for access to sunlight and forage. The animals were dewormed two times within the experimental period.

The actual experimental period lasted for complete 70 days.

Routine inspection for cleanliness was carried out. Freshly cut forage consisting of *Panicum maximum, Aspilia africana, Pennisetum purpureun* (Elephant grass) was fed as basal diet and a concentrate ratio of Grower Mash was used as supplement.

The animals were fed twice daily, in the morning and evening. Salt lick was provided as mineral supplement. Water was given *ad libitum* to the animals.

Experimental Design and Drug Administration

The twelve Balami rams were divided into 3 experimental groups consisting of 4 animals per group with one animal per replicate in a Completely Randomized Design (CRD).

These groups were assigned to 3 levels of Diclair® as treatments. The levels of Diclair® were 0.00 i.u, 81.00 i.u and 162.00 i.u represented as T_1, T_2 and T_3 respectively. T_1, which contained no Diclair® served as the control. The rams were treated by intramuscular injection.

The injections were given as follows: Diclair® was supplied in 7 vails, each vial containing FSH 75 i.u and LH 75 i.u. The content of the first vial was dissolved in 1mL of physiological saline solution immediately prior to use, resulting in a solution of DFSH 75 i.u plus DLH 75 i.u per mL.

All treatments were administered intramuscularly on the hind leg (thigh) of each ram using a one mL syringe with 0.01mL graduation (Table 1 and 2).

Table 1. Doses of Diclair® Administered to Mature Balami Rams

Day	Treatment Dosage (mL)		
	T_1	T_2	T_3
1	0.00	0.12	0.24
2	0.00	0.12	0.24
3	0.00	0.12	0.24
Total	**0.00**	**0.36**	**0.72**

Table 2. Concentration of Diclair® on Mature Balami Rams

Day	Concentration of Diclair (i.u)		
	T_1	T_2	T_3
1	0.00	18.00	36.00
2	0.00	18.00	36.00
3	0.00	18.00	36.00
Total	**0.00**	**54.00**	**108.00**

Semen Collection and Evaluation

Semen collection was done by electro–ejaculation method [NOAKES *et al.*, 2001] after one week of Diclair® administration and continued at 2 weeks interval for 9 weeks. The semen was collected between 8.00 am and 9.00 am.

This was to ensure that quality semen would be obtained. A transparent graduated tube immersed in a protective jacket containing water at 37°C, with a funnel was used to collect the semen.

The animal's hind limbs were held up by an assistant, with shoulder and fore limbs of the animal held tightly between the two legs of the assistant.

The Vaseline lubricated probe was inserted gently into the rectum.

The rhythmic stimulation of the ampullae and sacral nerve plexus caused erection and subsequently ejaculation within few minutes. Semen evaluation was done as promptly as possible post collection as described by Rodriguez–

Mertinez and Barth for qualitative and quantitative parameters such as semen volume, semen pH, sperm concentration, sperm motility, live sperm percentage, dead sperm percentage [RODRIGUEZ–MERTINEZ and BARTH 2007].

Hormonal Assay

Blood sample (5 mL each) were obtained with needle and syringe by jugular vein puncture of the on day 7 after the Diclair® injection for testosterone, FSH and LH evaluation. It was cooled immediately in iced water and transferred to the laboratory, refrigerated at 4°C for 1 hour and the serum separated by centrifugation at 5,000 rpm for 10 minutes. The serum was stored immediately at 20°C until enzyme immune assayed (EIA) for testosterone, FSH and LH as described by Micallef and collab. [MICALLEF *et al.* 1995].

Biochemical constituents of Seminal Plasma

Semen samples used for estimation of biochemical constituents of seminal plasma were centrifuged at 15,000rpm for 15 minutes.

The seminal plasma samples were immediately subjected to laboratory analysis for the following biochemical parameters: urea, glycerophos–phocholine, ascorbic acid, sodium, potassium, bicarbonate and fructose.

Sodium and potassium concentrations were estimated with a flame photometer on samples suitably diluted with deionized water, while bicarbonate and urea concentrations were determined according to the method of Baker and Silverton [BAKER and SILVERTON 1986].

Fructose concentration in the plasma was determined according to the procedure of Singgh [SINGGH, 2004].

Glycerophosphocholine and ascorbic acid concentrations were determined according to the method of Robert and collab. [ROBERT *et al.* 2000].

Data Analysis

Data obtained on semen characteristics, hormonal assay and seminal plasma constituents of Balami rams were subjected to analysis of variance (ANOVA) using the technique of Steel and Torrie [STEEL and TORRIE 1980].

Significant treatment means were separated using Duncan's New Multiple Range Test as described by Obi [OBI, 1990].

Results and discussion

The results of Diclair® administration on semen characteristics of Balami rams are shown in Table 3.

Table 3.

Effect of Diclair® on Semen Characteristics of mature Balami Rams

	Treatment (Diclair® i.u)			
Parameters	T_1 0.00	T_2 54.00	T_3 108.00	SEM
Semen volume (mL)	0.75^{c}	2.05^{a}	1.55^{b}	0.24
pH	8.25	8.50	8.00	0.17
Individual Motility (%)	45.50^{c}	80.50^{b}	90.50^{a}	8.63
Sperm concentration ($x10^6$/mL)	0.99^{a}	1.06^{a}	0.93^{b}	0.04
Proportion of live sperm cells (%)	65.50^{b}	79.00^{ab}	89.50^{a}	6.95
Proportion of normal sperm cells (%)	90.50^{b}	92.50^{b}	94.50^{a}	0.16
Proportion of dead sperm cells (%)	34.50^{a}	21.00^{ab}	10.50^{b}	6.95

$^{ab:}$Means within row having different superscript are significantly (P<0.05) different.
SEM = Standard error of means.

There were significant differences (P<0.05) among the treatment groups in semen volume, individual motility, sperm concentration, percentage live sperm cells, percentage normal sperm cells and percentage dead sperm cells.

However, semen pH was similar (P > 0.05) among the treatment groups.

Rams on T_2 recorded the highest value of 2.05ml in semen volume and this differed significantly (P<0.05) from rams on the control treatment (T_1) and T_3 which were also significantly different (P<0.05) from each other in semen volume.

The lowest value in semen volume was observed in rams on T_1 (0.75 mL).

The highest semen volume obtained in this study (2.05 mL) was higher than the mean ejaculate volume of 1.25 $\pm$ 0.7 mL reported by Iheukwumere

and Okere [IHEUKWUMERE and OKERE 1990] in Yankasa rams of similar ages. Iheukwumere and collab. [IHEUKWUMERE *et al.* 2008] noted that methods of semen collection, season of the year, breed, and age, body weight of animal, scrotal circumference and frequency of semen harvest can affect ejaculate volume in rams.

There were no significant differences (P>0.05) among the treatment groups in semen pH. Rams on T_2 recorded the highest numerical value of 8.50 in semen pH. The lowest numerical value in semen pH was observed in rams on T_3 (8.00). Semen pH values obtained in this study were within the normal range of 7–8 reported by Meacham [MEACHAM, 2002] except rams on T_2 whose semen pH (8.50) was slightly higher than the normal range. The measured pH depends on the length of time since ejaculation and it tends to increase shortly after ejaculation as a result of loss of CO_2 [MEACHAM, 2002].

Rams on T_3 recorded the highest score of 90.50% in individual motility and this differed significantly (P<0.05) from rams on T_1 and T_2 which were also significantly different (P<0.05) from each other in individual motility.

The lowest score individual motility was observed in rams on T_1 (45.50%).

The highest score in individual motility obtained in this study (90.50%) was higher than the score of 85.0 ± 7.5% reported by Iheukwumere and collab. [IHEUKWUMERE *et al.* 2001] in Yankasa rams of similar ages. Sperm motility is affected by frequency of semen collection [IHEUKWUMERE *et al.* 2008, BUTU, *et al.*, 2014].

Rams on T_2 recorded the highest value of 1.06 x 10^6/mL in sperm concentration and this differed significantly (P<0.05) from rams on T_3. There was no significant different (P>0.05) between rams on T_2 and those on T_1 in sperm concentration.

The lowest value in sperm concentration was observed in rams on T_3 (0.93×10^6/mL).

The sperm concentration values obtained in this study were higher than the highest value for sperm concentration 0.79±0.07 ($\times10^{10}$/mL) reported by Iheukwumere and collab. [IHEUKWUMERE *et al.* 2001] in Yankasa rams of similar ages.

This was within the normal range of 2000 to more than 1,000 million spz/mL reported Rodriguez–Martinez [RODRIGUEZ–MARTINEZ, 2003]. Normally an increase in the semen collection frequency is associated with a decrease in sperm concentration [AROITA *et al*., 2000; IHEUKWUMERE and OKERE, 1990].

Rams on T_3 recorded the highest percentage of live spermcells (8.9–50%) and this differed significantly, (P<0.05) from rams on the control treatment which was similar (P>0.05) to rams on T_2 in percentage live sperm cells.

However, there was no significant difference (P>0.05) between rams on T_3 and T_2 in percentage of live sperm cells. The lowest percentage of live sperm cells was observed in rams on the control treatment (65–50%).

The highest percentage of live sperm cells obtained in this study was higher than the range of 77.2±9.7–79.4±4.9% reported by Iheukwumere and collab. [IHEUKWUMERE *et al.* 2001] in Nigerian Yankasa rams. Rams on T_3 recorded the highest percentage of normal sperm cells 94.50% and this differed significantly (P< 0.05) from rams on T_1 and T_2which were also significantly different (P<0.05) from each other in percentage of normal sperm cells.

The percentages of normal sperm cells obtained in this study were within the range of 82.4±3.8 – 87.0±8.2% reported by Iheukwumere and collab. [IHEUKWUMERE *et al.* 2001] in Nigerian Yankasa rams.

Rams on T_1 recorded the highest percentage of dead sperm cells (34.50 %) and this differed significantly (P<0.05) from rams on T_3 which was similar (P>0.05) to rams on T_2. There was no significant difference (P>0.05) between rams on T_1 and T_2 in percentage of dead sperm cells. The lowest percentage of dead sperm cells was observed in rams on T_3 (10.50%).

The observation in this study that the group that received higher (0.72 mL) dose of the test drug recorded the highest percentage of live sperm cells, normal sperm cells and lowest percentage of dead sperm cells suggests that a high

dose of the drug such as 0.72 mL/ram within 3 days given in this study could have high capacity for induction of sperm production, improvement of semen quality and have no deleterious effects on sperm cells.

The result of Diclair® administration on hormonal profile of Balami rams are shown in Table 4.

There were significant differences (P<0.05) among the treatment groups in all the hormonal parameters measured: follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone. Rams on T_2 recorded the highest value of 10.24 (iu/L) in FSH and this differed significantly (P<0.05) from rams on T_1 which were similar (P>0.05) to rams on T_3 in FSH values. There was no significant difference (P>0.05) between rams on T_2 and T_3 in FSH values.

The lowest value in FSH was observed in rams on the control treatment (T_1) (9.73 iu/L). The observation in this study that the FSH value in the rams treated with 0.36mL Diclair® was numerically higher than in the group that received higher dose of the drug suggest that a high dose of the drug such as 0.72 mL/ram within 3 days given in this study could excite suppressive effect on the hypothalamus.

Rams on T_1 recorded the highest value of 11.17 (iu/L) in LH and this differed significantly (P < 0.05) from rams on T_2 which had 6.89 (iu/L). However, there was no significant difference (P>0.05) between rams on T_1 and T_3 in LH values. The lowest value in LH was observed in rams on T_2 (6.89 iu/L).

The observation in this study that the LH value in rams on the control treatment was higher (P < 0.05) than in the group that received Diclair® treatment suggest that the doses of the drug given in this study; 0.36 mL, and 0.72 mL per ram within 3 days could excite suppressive effect on the hypothalamus.
It is common knowledge that LH as interstitial cell stimulating hormone (ICSH) stimulates the interstitial cells of leydig to produce testosterone which facilitates the process of spermatogenesis [HERBERT *et al.*, 2000, RODINO, *et al.*, 2014, BUTU, *et al.*, 2015,].

Table 4.

Effect of Diclair® on Hormonal Profile of Mature Balami Rams

	Treatment (Diclair® i.u)			
Parameters	T_1 0.00	T_2 54.00	T_3 108.00	SEM
FSH (iu/L)	9.73^{b}	10.24^{a}	10.10^{ab}	0.15
LH (iu/L)	11.17^{a}	6.89^{b}	11.08^{a}	0.57
Testosterone (ng/mL)	9.87^{ab}	6.78^{b}	12.05^{a}	1.53

$^{abc:}$Means within row having different superscript are significantly (P<0.05) different.
SEM = Standard error of means.

Rams on T_3 recorded the highest value of 12.05 (ng/mL) in testosterone and this differed significantly (P<0.05) from rams on T_2 which had 6.78 (ng/mL).

However, there was no significant difference (P>0.05) between rams on T_3 and T_1 in testosterone values. Rams on T_2 was similar (P>0.05) to those on T_1 in testosterone values.

The lowest value in testosterone was observed in rams on T_2 (6.78 ng/mL). The testosterone values obtained in this study were within the normal range of 0–20 ng/mL reported in sheep by King and collab. [KING *et al.* 1993].

The results of Diclair® administration on biochemical constituents of seminal plasma of Balami rams are shown in Table 5.

There were significant differences among the treatment groups in all the biochemical constituents of the seminal plasma measured: urea, glycerophosphocholine, ascorbic acid, sodium, potassium, bicarbonate, and fructose. Rams on T_3 recorded the highest value of 41.35 (mg/100 mL) in seminal plasma urea and this differed significantly (P>0.05) from rams on T_1 which were similar (P > 0.05) to rams on T_2 in urea level in the seminal plasma.

There was no significant difference (P > 0.05) between rams on T_3 and T_2 in urea level in the seminal plasma.

The lowest value for urea in seminal plasma was observed in rams on T_1 (34.15 mg/100 mL). The values for urea in seminal plasma obtained in this study were lower than the normal range of 43–75 mg/100mL reported by Roller and collab. in rams [ROLLER *et al.* 1982]. However, the values for urea in this study were within the range of 17–42mg/dl reported by Kaneko [KANEKO 1980] in rams.

Table 5.

Effect of Diclair® on Biochemical Constituents of Seminal Plasma of Balami Rams

	Treatment (Diclair® i.u)			
Parameters	T_1 0.00	T_2 54.00	T_3 108.00	SEM
Urea (mg/100mL)	34.15^{b}	39.50^{ab}	41.35^{a}	2.16
Glycerophospho–cholin (mg/100mL)	920^{b}	925^{b}	1020^{a}	26.59
Ascorbic acid (mg/100mL)	5.65^{b}	7.00^{ab}	7.60^{a}	0.58
Sodium (mmol/L)	43.30^{b}	44.05^{ab}	47.16^{a}	1.18
Potassium (mmol/L)	4.00^{b}	5.70^{ab}	5.80^{a}	0.58
Bicarbonate (mmol/L)	22.10^{b}	24.00^{ab}	25.30^{a}	0.93
Fructose (mmol/L)	450.00^{b}	470.00^{ab}	480.00^{a}	8.83

^{ab}:Means within row having different superscript are significantly (P<0.05) different.
SEM = Standard error of means.

Cortada and collab. reported that sharp increase in plasma urea level could result in gonadal degeneration and infertility, with reduced sperm production and loss of libido [CORTADA *et al.* 2000].

Rams on T_3 recorded the highest value of 1020 mg/100 mL in glycerophosphocholine (GPC) in the seminal plasma and this differed significantly (P<0.05) from rams on T_1 and T_2 which were similar (P>0.05) to each other in glycerophosphocholine values.

The lowest value in glycerophosphocholine was observed in rams on T_1 (920 mg/100mL). Burt and Ribolow [BURT and RIBOLOW, 1994] reported that in rams, glycerophosphocholine reaches extremely high level in semen, the highest being a remarkable 100 millimolar.

In men, low level of GPC in the semen correlate with poor sperm motility [HAMAMAH, 1998]. Poor GPC status has been linked to male infertility in human males.

Rams on T_3 recorded the highest value of 7.60(mg/100mL) in ascorbic acid in the seminal plasma and this differed significantly (P<0.05) from rams on T_1 which were similar (P>0.05) to rams on T_2 in Ascorbic acid levels.

There was no significant difference (P>0.05) between rams on T_3 and T_2 in Ascorbic acid level in the seminal plasma.

The lowest value in Ascorbic acid was observed in rams on T_1 (5.65 mg/100 mL). Studies have shown that vitamin C plays a vital role in enhancing sperm motility, keeping sperm strong by protecting them from free radicals (www.malefertilitysupplements.com.2013).

Rams on T_3 recorded the highest value of 47.16 (mmol/L) in seminal plasma sodium and this differed significantly (P<0.05) from rams on T_1 which were similar (P > 0.05) to rams on T_2 in sodium value. There was no significant difference (P > 0.05) between rams on T_3 and T_2 in sodium values.

The lowest value in seminal plasma sodium was observed in rams on T_1 (4.00 mmol/L). Sodium bicarbonate in seminal plasma stimulates sperm motility [OKAMURA *et al.*, 2006].

Rams on T_3 recorded the highest value of 5.80 (mmol/L) in seminal plasma potassium and this differed significantly (P<0.05) from rams on T_1 which were similar (P > 0.05) to rams on T_2.

There was no significant difference (P>0.05) between rams on T_3 and T_2 in potassium values. Potassium is known to maintain sodium levels and facilitate blood circulation and flow (www.howtoincreasespermcount.org.2013).

Rams on T_3 recorded the highest value of 25.30 (mmol/L in seminal plasma bicarbonate and this differed significantly (P<0.05) from rams on T_1 which were similar (P > 0.05) to rams on T_2 in bicarbonate values. There was no significant difference (P>0.05) between

rams on T_3 and T_2 in bicarbonate values. Bicarbonate values obtained in this study were higher than the mean value of 20.00 (mmol/L) reported by Okamura and collab. [OKAMURA *et al.*, 2006] who also inferred that sodium bicarbonate in seminal plasma stimulates sperm motility.

Rams on T_3 recorded the highest value of 480.00 (mmol/L) in seminal plasma fructose and this differed significantly ($P<0.05$) from rams on T_1 which were similar ($P>0.05$) to rams on T_2 in fructose values. There was no significant difference ($P>0.05$) between rams on T_3 and T_2 in seminal plasma fructose values.

Owen and Katz [OWEN and KATZ, 2005] reported that fructose level is a measure of seminal vesicle function being a source of energy for the sperm.

Fructose values obtained in this study were within the range of 136–628mg/100mL for seminal plasma fructose reported by [GONZALES *et al.*, 1993].

Conclusions

The results of this study showed that Diclair® improved semen quality and Biochemical constituents of the seminal plasma at the level of 108.00 i.u without any deleterious effects on hormonal profile. Though the hormonal levels fall within the normal ranges for adult sheep, the variations observed suggest the need to constantly monitor hormonal profile of Balami rams under Diclair® treatment for sperm and semen production.

References

1. Abu, A.H.; Ameh, M.; Iheukwumere, F.C.; Semen quality of Nigerian local cock treated with human menopausal gonadotrophin (Pergonal®). *Livestock Research for Rural Development*, **2006**, 18, Article #44.
2. Ameh, M. Effect of Pergonal® on semen quality, haematological values and carcass characteristics of the Nigerian Local cocks. M.Sc. Thesis Department of Animal Science and Fisheries Abia State University, Umuahia Nigeria. **2004**.
3. Anonymous, www.howtoincreasespermcount.org. **2013**.
4. Anonymous, www.malefertilitysupplements.com. **2013**.
5. Aroita, Z.; Faketo, M.V.; martin–Rill–O;S.; Dealba, C.; Moreno, C.; Cludad, M.J.; Rafel, O.; Effect of collection frequency on production, quality and storage of Young Bucks Semen. *7th World Rabbi Congress, Valencia, Vol. A*. **2000**.
6. Baker, F.J.; Silverton, R.F. Introduction to Medical Laboratory Technology, *6th Edn. Butter–Worth England*. **1986**.
7. Burt, C.T.; Ribolow, H. Glycerol phosphorylcholine (GPC) and serine ethanolamine phosphodiester (SEP): evolutionary mirrored metabolites and their potential metabolic roles. *Comparative biochemistry and physiology.* **1994**; 108B: 11.
8. Butu, A.; Rodino, S.; Golea, D.; Butu, M.; Butnariu, M.; Negoescu, C.; Dinu–Pirvu, C.E. Liposomal nanodelivery system for proteasome inhibitor anticancer drug bortezomib, *Farmacia*. **2015**, 63(2), 224–229.
9. Butu, M.; Butnariu, M.; Rodino, S.; Butu, A. Study of zingiberene from *Lycopersicon esculentum* fruit by mass spectometry, *Digest journal of nanomaterials and biostructures*, **2014**, 9(3), 935–941.
10. Cortada, C.N.M.; Lucci, C.O.; Gonazalez, R.A.F.; Valentin, R.; de mattos, C.B. Plasma Urea levels on Reproductive Parameters of wool–less Rams (*Ovis aries, linnaeus*, 1958). *Brazilian Journal of Veterinary Research and Animal Science*. Sqo Paulo dez, **2000**, 37(6).
11. Dixon, T.A.; Hopkins, G.J. Super ovulation in cattle using porcine pituitary Gonadotrophin preparation (Phisset Serono) in: *Plusset Scientific Literature Serono Veterinary*, *Bome Italy*, **1996**, pp. 22–23.
12. Gonzles, G.F.; Kortebani, F.; Mazzolli, A.B. Hyper viscosity and hypo function of the seminal vesicles, *Archives of Andrology*. **1993**. 30, 63–68.
13. Hamamah, S. Quantification by magnetic resonance spectroscopy of metabolites in seminal plasma able to differentiate different forms of azoospermia. *Human Reproduction.* **1998**; 13, 132.
14. Herbert, U.; Okoro, P.; Umesiobi, D.O.; Iloeje, M.U. Effects of two preparations of clomiphene citrate on

the superovulation of West African Dwarf Ewes. *14th Int. Congr. On Anim. Reprod. Sweden*. **2000**, 2:114.
15. Iheukwumere, F.C.; Abu, A.H.; Ndubuisi, E.C. Effect of FSH + LH (Pergonal®) Treatment and Serum Metabolites of West African Dwarf Goats. *Journal of Animal and Veterinary Advances,* **2008**. 7(1), 46–50.
16. Iheukwumere, F.C.; Herbert, U.; Umesiobi, D.O. Biochemical Evaluation of seminal plasma in Yankasa Rams under different Intensities of Semen Collection, *International Journal of Agriculture and Rural Development.* 2001, 2:29–34.
17. Iheukwumere, F.C.; Okereke, C. Effects of frequent ejaculation on semen characteristics of Nigerian Yankasa Rams. *Small Ruminant Research*, **1990**, 3, 77–83.
18. Kaneko, J.J.; Harvey, J.W.; Bruss, M.I. Clinical Biochemistry of Domestic Animals, *5th Edition Academic press. San Diego Carliffonia P*. **1997**, 885–905.
19. King, G.I.; Nelmann–Sorenson, A.; Tribe, D.E. Reproduction in Domesticated Animals 9th Edition World Animal Science B. Disciplinary Approach. *Elsevier science Publishers, London New York*, **1993**. p. 106.
20. Meacham, R. Perspectives and Editorials. Andrologia. *Asian Journal of Andrology*, **2002**. 23, 330–331.
21. Micallef, I.A.; Hays, M.M.; Latif, A.; Alhasan, R.; Sufi, S.B. Serum binding of steroid Tracer's and its possible effects on Direct steroid immune assay. *Annals of clinical biochemistry*. **1995**. 32, 566–574.
22. Noakes, D.E.; Parkinsom, T.J.; Gary, C.W. Veterinary Reproduction and Obstetrics, *England 8th Edition*. **2001**.
23. Obi, I.U. Statistical methods of detecting differences between treatment means. *Snap press 2nd Ed. Enugu. Nigeria*, **1990**. 24–35.
24. Okamura, N.S.; Tajima, Y.; Soejimaj, A.; Masudap, H.; Sugita, Y. Sodium Bicarbonate in seminal plasma stimulates the motility of Mammalian spermatozoa through Direct Activation of Adenylate cyclase. *The Journal of Biochemical Chemistry,* **2006**; 9699–9705.
25. Oni, O.O. Breeds and Genetic Improvement of small ruminant in: Manual for small ruminant Production in Nigeria: A training workshop on small ruminant production held at the National Animal Production Research Institute Zaria, Nigeria 13–18 January, **2002**. 1–7.
26. Owen, D.H.; Katz, D.F. Review of the physical and chemical properties of Human Semen and the formulation of a semen stimulant. *Journal of Andrology,* **2005**. 26(4).
27. Robert, K.M.; Daryl, K.G.; Peter, A.M.; Victor, W.R. Mayers Biochemistry, *25th Edn. McGraw Hill. New York*, **2000**. 763–765.
28. Rodino, S.; Butu, M.; Negoescu, C.; Caunii, A.; Cristina, R.T.; Butnariu, M. Spectrophotometric method for quantitative determination of nystatin antifungal agent in pharmaceutical formulations, *Digest journal of nanomaterials and biostructures*, **2014**, 9(3), 1215–1222.
29. Rodriguez–Martinez, H. Laboratory Semen Assessment and Prediction of Fertility; Still Utopia? *Reproduction in Domestic Animals*, **2003**. 38, 312–318.
30. Roller, M.M.; Riedmann, G.S.; Romkema, G.E.; Swanson, R.N. Ovine Blood Chemistry values measured during Ammonia toxicosis. *American Journal of Veterinary Research*, **1982**. 43(6). 1068–71.
31. Singgh, S.P. Practical Manual in Biochemistry (5th ed.) Satish Kuma Jain, India, **2004**. 203–255.
32. Steel, R.G.D.; Torrie, J.H. Principles and procedures of Statistics. A. *Biometric Approach 2nd Ed. Mc. Graw–Hill Book, Co. Inc. New York*. **1980**.

Received: January 07, 2016
Article in Press: April 26, 2017
Accepted: Last modified on: May 20, 2017