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Original article

Effect of "inhibin" from bovine testicular and follicular fluids on attainment of puberty in male rabbits

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ABSTRACT

For this study, 15 young male rabbits of same breeds, with age range of 11-12 weeks old and weighing 1.0 ±0.2kg were used. These were randomly assigned to three treatments, each consisting of five rabbits per treatment. The treated groups(Treatments B and C) and the control group (Treatment A)were injected intramuscularly with charcoal-extracted bovine follicular fluid (FF), charcoal extracted bovine testicular fluid (TF) and charcoal treated distilled water (DW) respectively, at the rate of 0.2ml per rabbit on every other day and on three different occasions. Duration of the treatment lasted six days. Data were analyzed using descriptive statistics and ANOVA. After administration of the different treatments, body weight (BW), testes weight (TW) and histometric studies (HS) were conducted. Histometric studies included: seminiferous tubule diameter (STD), cellular elements (CE), stages of the cycle of seminiferous epithelium (CSE), determination of daily sperm production (DSP) and daily sperm production per gram testis (DSP/g). Figures obtained pertaining to their weights showed that they grew significantly over their initial weights with animals treated with TF showing highly significant ($p<0.01$) weight increase of 0.78kg, which on the average represents 110.8% and 95% higher in weight gain than those of FF and control groups. There were no significant differences ($p>0.05$) in paired TW among the three different groups. STD of control group was significantly ($p<0.01$) higher (203.46µ) than those of the treated groups. There were no significant ($p>0.05$) differences among the three different treatments in the frequency occurrence of stages 2 and 7 of the CSE, DSP and DSP/g testes. However, the frequency of

occurrence of stages 1, 4 and 8 of the CSE differ significantly (*p<0.01*) between control group and treated groups. There was a highly significant spermatozoa decrease (*p<0.01*) in the treated groups when compared with control group and this supports the ability of the inhibin from bovine follicular and testicular fluids to inhibit the onset of active spermatogenesis.

1. Introduction

Puberty is the developmental stage in an animal when physiological reproductive activities starts, and this is initiated by the hormone estrogen in the female and this brings about such activities like egg development, ovulation, heat behavior and inter - uterine development (Ekeocha, 2009). As for the male, activities are brought about by the hormone testosterone and this initiates such activities like spermatozoa development, the growing urge to mate known as libido and the development of accessory sex organs and when all these activities have reached the optimum stage then, the animal is said to be sexually matured (Ekeocha, 2009). Sexual maturity comes after puberty attainment. All these developments are derived from the maturation of the sex organs and the accompanying surge in the secretion of sex hormones. The time of onset and the age and weight at which puberty is attained vary greatly. Factors affecting the onset of puberty include inheritance and genotype, geographical location, diseases and socio-economic factors especially as they affect nutrition and general well-being (Anderson, 1974). These factors account for the various figures recorded by researchers (Amann, 1972). It can also be postulated that the mechanism involved in the onset of puberty depends on a critical ratio of stimulatory and inhibitory factors that must be established before puberty can be initiated. In the male, maturity can only be arbitrarily regarded as a moment in the continuum of testicular growth and changes in secondary sexual characters (Fox, 1968 and Vanderbergh, 1968).

Male puberty can also be determined by measuring testicular development and electro-ejaculation. An attempt to present a suitable technique for evaluating male sexual maturity with ease and without any injury on the animal was made by Vanderbergh (1971). It involves taking penile smear of male by gently and carefully rotating a moistened cotton bud between the orifice and the glan penis, smearing the adherent material on to a glass slide and examining the slide with staining for the presence of spermatozoa which is taken as an index of puberty (Vanderbergh, 1971). Courot (1978) suggested that follicular stimulating hormone (FSH) could be the main gonadotropin in the prepubertal period while Lostroh (1969) stated that FSH and luteinizing hormone (LH) may act in synergistic way. On the basis of cultures of seminiferous tubules and experimental evidence from immature rats, Steinberger (1972) suggested that no hormones were required for spermatogenesis to proceed from the early spermatogonia steps up to those of late pachytene and that FSH and testosterone would be necessary only for the final stages of the cycle. Information on the development of male sexual maturity has received less attention since there has not been any definite index of sexual maturity (Vanderbergh, 1971). Breeders are looking for ways of manipulating reproductive events with a view of hastening puberty. With this in mind, a study was designed to entail the determination of the effect of inhibin obtained from bovine follicular and testicular fluids on the attainment of puberty in immature male rabbits.

2. Materials and methods

2.1. Experimental animals and management

15 pre-pubertal male rabbits of same breeds were used in this experiment. They averaged 1.0 $\pm$ 0.2 kg body weight with age range of 11-12 weeks. The rabbits were housed in a well-ventilated standard rabbit house with ant traps built round to prevent the entry of soldier ants. Predators were prevented with the aid of wire netting. The house, drinking and feeding troughs were cleaned and disinfected one week prior to the date of arrival of rabbits. Normal vaccination of rabbits against mange and ectoparasites Izomech® was carried out and fed commercial rabbit feed (growers mash, pellet form) with *Centrosena pubescens* supplied *ad libitum*. Water was also free

choice. Their water troughs were washed and sterilized every other day to prevent the occurrence of any fungal disease. The same feed was fed one week before the onset of the experiment to acclimatize the rabbits and the experiment started at the 13th week of age when the animals were randomly assigned to three groups with five animals per group. The three groups were

1. Control "Charcoal distilled water"
2. Bovine follicular fluid treated group
3. Bovine testicular fluid treated group.

2.2. Follicular fluid collection

The bovine follicular fluid used in this study was obtained by aspirating ripe follicles from cow ovaries within four hours of slaughtering at Bodija Abattoir in Ibadan. The follicular fluid was stored at -20^{o}C in sterilized specimen bottles until charcoal extraction to remove steroids.

2.3. Testicular fluid collection

The bovine testicular fluid used in this study was obtained by removing the testicular parenchyma from bull's testes after few hours of slaughtering at Bodija Abattoir. In order to obtain the testicular fluid, a known amount of testicular parenchyma was blended in a sterile blender with physiological saline (buffer solution) added at a concentration of 1ml (2.5g of testicular parenchyma). This was poured into sterile test tubes and centrifuged at room temperature for 15 minutes at 3000rpm to remove the cells. The fluid was then decanted and stored at -20^{o}C until charcoal extraction to remove steroids.

2.4. Charcoal extraction

A known volume of each follicular and testicular fluid sufficient for all injections was mixed separately in sterile beakers with activated charcoal at a concentration of 5mg/ml of fluid to remove steroids (Welscher *et al.*, 1997; Miller *et al.*, 1979).

These were agitated at room temperature using vortex mixing for 20 minutes and then centrifuged at room temperature for 15 minutes at 3000rpm to remove all particles. The supernatant was collected and stored at -20^{o}C in wire packs. After charcoal treatment, 99% of the original steroids had been removed. A known volume of distilled water was also treated as above to serve as the control.

2.5. Dilution process

After charcoal extraction, the supernatant both from follicular and testicular fluids were diluted at the ratio of 1:1; that is, 1ml of follicular or testicular fluid/1ml of distilled water to reduce its concentration.

2.6. Experimental design

The experimental design was a completely randomized design. 15 male rabbits were grouped randomly into the treated and control groups, each group consisting of 5 male rabbits. The treated groups were injected intra-muscularly with charcoal extracted follicular or testicular fluids at the rate of 0.2ml per rabbit on every other day on three different occasions. The control group was injected similarly with 0.2ml of charcoal extracted distilled water.

2.7. Collection of penile smears

Penile smears were taken by gently and carefully rotating a moistened cotton bud between the orifice and the glan pennis, smearing the adherent material unto a glass slide and examine the slide with staining for the presence of sperm cells. When 50% of the smears from the male rabbits were positive, the rabbits were weighed and finally slaughtered by decapitation.

2.8. Testicular histology

Animals were killed by dislocation of the neck after which the endocrine organs (pituitary, adrenal, and kidney) as well as the reproductive tracts were removed and wrapped with foil paper and weighed on electrochemical balance. The reproductive organs involved were testis and epididymides. Immediately after removal, each epididymis was dissected and a puncture made on the caudal section. The exudates was smeared onto a clean glass slide with a drop of distilled water and examined under the microscope (X40) for the presence of

sperm cells. Both the testes and epididymis were sampled and fixed in aqueous Bouin's fixative and processed routinely for histology.

2.9. Dehydration and embedding

After fixing in Bouin's fixative for 6hrs, the tissues samples were rinsed in 70% ethyl alcohol (EtOH) and thereafter dehydrated in increasing concentrations of ethyl alcohol and then cleared in three changes of chloroform as follows:

70% EtOH → 80% EtOH → 90% EtOH → 95% EtOH ↓

$CHCl_3$ ← $CHCl_3$ ← 100% EtOH ← 100% EtOH ← 95% EtOH

↓

$CHCl_3$ → paraffin

Each sample was allowed 45 minutes in each alcohol and chloroform change and was left overnight in paraffin at (mp 60^{o}C). The cleared tissue samples were impregnated under pressure for 30 minutes and embedded in wax to form tissue blocks. Histological sections 5 microns thick were floated and flattened out on 40^{o}C water and then picked up carefully with clean slides smeared with Mayer's egg albumin. These slides were stored in air incubator for 30 minutes after which they were stained with Haematoxylin and Eosin (H and E).

2.10. Shrinkage of testicular tissue

Shrinkage of testicular tissue due to fixation, dehydration, clearing and paraffin impregnation was determined. Tissue samples about the size used for the experiment were weighted. Therefore, their densities were determined having obtained their volumes by Archimedes principle of water displacement. These samples were thereafter treated exactly as the experimental samples and processed according to the same histological methods. After paraffin impregnation the volumes of these tissue samples were again determined by using xylene instead of water. Correction for tissue shrinkage was calculated as below:

$$\text{Correction for tissue shrinkage} = \frac{\text{Initial volume} - \text{Final volume}}{\text{Initial volume}}$$

The two slides per rabbit were then evaluated. The prepared slides were analyzed for each of the two treatments including the control.

2.11. Histometry

2.11.1. Volumetric proportions of spermatogenic elements

The volumetric proportions of spermatogenic elements in the seminiferous epithelium were determined using the method of Chalkley (1943). A twenty five point ocular graticule (CarlZeiss, Oberkochen) was used in this estimation. Microscopic fields were observed at random and the structures, including artifacts, under 'hit' were recorded. Precaution was taken to insure that no field was examined twice; fifty fields were examined giving a total of 1,250 hits and total hits of 2,500 per slide since there were 2 tissues per slide. All observations were made on the 5-micron sections using an x8 occular and a 100x oil immersion objective.

Testicular elements classified included:

a. Spermatogonia A-type
b. Spermatogonia B-type
c. Young primary spermatocytes:
   i. Preleptotene primary spermatocytes
   ii. Leptotene primary spermatocytes
   iii. Zygotene primary spermatocytes
d. Old primary spermatocytes:
   i. Pachytene Primary spermatocytes
   ii. Diplotene Primary Spermatocytes
e. Secondary spermatocytes
f. Round spermatids at stages $R_1 – R_3$
   iii. Elongating spermatids at stages el_4, $el_5 – el_8$

iv. Maturing or elongated spermatids at stages $L_9 – L_{10}$

g. Free spermatozoa
h. Sertoli cells
i. Lumen
j. Cellular cytoplasm
k. Intertubular space
l. Seminiferous tubules

The percentage of testis occupied by a particular element was estimated by the formula below.

$$\text{Volume \% of element} = \frac{100 \text{ (Total 'hits' on the element)}}{\text{Total possible 'hits'} – \text{No. of artifact 'hits'}}$$

2.11.2. Relative duration of the stages of the cycles of seminiferous epithelium

The relative duration or frequency of the eight stages of the seminiferous epithelial cycle was determined by classifying the stages of the cycle that occurred in (100) one hundred cross-sections of the seminiferous tubules observed microscopically at a X40 objective based on the criteria used by Ortavant (1959) and Amann (1962) and utilized by Swierstra (1966).

The frequency of occurrence of each of the eight stages in the cycle of the seminiferous epithelium was recorded in 50 tubules on each tissue and 100 tubules on each slide giving a total of (200) two hundred tubules per rabbit. The frequency was calculated on percent basis. The relative duration or occurrence of each stage was calculated using the formula below:

$$\text{Relative duration of stage 1} = \frac{100 \text{ (Total number of stage 1)}}{\text{Total number of all stages}}$$

The outlined 8 stages of the seminiferous epithelium in the rabbit is as follows

Stage 1 – Extends from the end of the spermatozoa release into the lumen until the beginning of the spermatid nuclei elongation. It is characterized by the presence of spermatids with round nuclei only.

Stage 2 – Extends from the elongation of spermatid nuclei up to the formation of the bundles of spermatids. This is the phase of nuclear elongation of the spermatids.

Stage 3 – Extends from the formation of the first elongated spermatid bundles in the sertoli cell cytoplasm up to the first maturation divisions.

Stage 4 – Extends from the appearance of the first divisions to the disappearance of the second maturation divisions.

Stage 5 - Extends from the end of the last maturation divisions up to the appearance of dusty chromatin in the nuclei of the young spermatids. During this stage the latter have a small nucleus containing some karyosomes connected by a chromatin network.

Stage 6 – Extends from the appearance of the dusty chromatin in the young spermatids up to the migration of the bundles of elongated spermatids towards the lumen of the seminiferous tubules.

Stage 7 – Extends from the beginning to the end of the centripetal migration of the elongated spermatids towards the lumen.

Stage 8 **–** Extends from the end of the migration of the spermatids to their complete release as spermatozoa into the lumen.

2.11.3. Seminiferous tubule diameter

Tubular diameter of seminiferous tubules were determined by measuring twenty randomly deflected near round tubules per tissue giving a total of 40 randomly selected near round tubules per slide (80/Animal) using a Carl Zeiss binocular microscope with an ocular micrometer calibrated against a stage micrometer. Two measurements at right angles to each other were taken on each tubule and the mean recorded. The mean diameter of the seminiferous tubule of each animal was expressed in microns.

2.11.4. Determination of daily sperm production (DSP)

Daily sperm production per testis per animal was estimated through quantitative testicular histology using a formula outlined by Swierstra (1966) as follows:

$$DSP = \frac{(CTV)\,(\text{Volume \% of round spermatid nuclei in the testis})}{\left[\begin{array}{c}\text{Average volume per round}\\ \text{spermatid nuclei in the testis}\end{array}\right]\left[\text{life span on round spermatid in days}\right]}$$

CTV = Corrected Testis Volume and determined as follows:

$$CTV = \left[\left[\begin{array}{l}\text{Gross}\\ \text{Testis}\\ \underline{\text{Weight}}\\ \text{Testis density}\end{array}\begin{array}{l}\text{Tunica}\\ -\ \text{Albuginea}\\ \underline{\text{Weight}}\end{array}\right] - \begin{array}{l}\text{Volume \% of}\\ \text{Mediastinum}\end{array}\right] \times \left[\begin{array}{l}\text{Shrinkage}\\ \text{Correction}\\ \text{Factor}\end{array}\right]$$

$$\text{Shrinkage Correction Factor} = \frac{\text{Initial Volume} - \text{Final Volume}}{\text{Initial Volume}}$$

Gross testis weight is described below: Tunica albuginea weight was determined in two other animals and averaged 18% of gross testis weight. Testis density was obtained by the formula D=m/v, m = mass, v = volume. Volume of mediastinum, 1% of testis volume was used (Swierstra and Ranefeld, 1968) while 0.476 (Egbunike, 1979) was used as shrinkage correction factor.

For average volume of round spermatids, 400 round spermatids averaged 6.08 microns in the laboratory. However, the diameter of 6.12µ (Swierstra and Foote, 1963) was used in the formula: $1/6\pi D^3$ (Swierstra, 1966).

2.11.5. Body weight

Differences in body weights at the beginning and end of experiment were also determined.

2.11.6. Testes weights and endocrine organ weights

Testes were trimmed of epididymides. They were then weighed in grams. Endocrine organs i.e. pituitary, thyroid, adrenals and kidneys were removed and weighed.

2.11.7. Testes volume

Each testis was volumetrically determined by water displacement in a measuring cylinder where the volumes were obtained by difference in water level without testis and water level with the testis.

2.11.8. Testis density

The density of each testis was determined using the formula D = m/v where D is density, M is mass (weight) and V is volume. Testis density was calculated from testis weight and volume.

2.12. Statistical analysis

Statistics used was one way Analysis of Variance (ANOVA) and Duncan's new Multiple Range Test (DMRT) was used for mean separation (SAS, 1999).

3. Results

3.1. Mean body weight

The mean body weight of the animals before the commencement of the experiment was significant ($P<0.05$). At the end of the experiment (five weeks) mean final body weight was highly significant among the three different groups with animals injected with testicular fluid (TF) having the highest mean final weight of 1.64 (Table 1). The increase in weight from initial mean weight to final mean weight was highly statistically significant ($P<0.01$) among the three different groups with animals injected with TF having two-fold superiority in weight gain (0.78kg) than the other two groups. The weight increase in animals with TF injection was 110.8% and 95% higher in weight than those of the animals with (Follicular Injection) and Normal Saline (NS) injection "Control group" (Table 1).

Table 1
Body Weight and Organ Weights.

	Normal Saline (Control)	Testicular Fluid (TF)	Follicular Fluid (FF)
Tubular Diameter (microns)	203.46[a]	195.42[b]	197.13[c]
Daily Sperm Production (x 10^6)	2.06[a]	1.98[a]	2.024[a]
DSP/g testis (x10^6)	2.008[a]	1.942[a]	1.966[a]
Paired Testis Weight (g)	3.06[a]	3.36[a]	2.68[a]
Initial Body Weight (kg)	0.91[ab]	0.86[b]	1.0[a]
Final Body Weight (kg)	1.31[b]	1.64[a]	1.37[b]
Increase in body weight (kg)	0.4[b]	0.78[a]	0.37[b]
Paired kidney weight (g)	8.28[b]	10.00[a]	8.33[b]
Paired adrenal gland (g)	0.6[b]	0.48[b]	0.76[a]
Paired thyroid gland (g)	0.12[a]	0.16[a]	0.17[a]
Pituitary (g)	0.02[a]	0.01[a]	0.02[a]

[abc]Means in the same row with different superscripts are significantly different (P<0.05).

3.2. Paired adrenal gland weight

There were highly significant difference (P<0.01) in the paired adrenal gland weight in animals among the three different groups with follicular fluid (FF) treated groups having the highest mean weight (0.76g) on the average which represent 26.7% and 58.3% increase in weight above control and TF treated groups.

3.3 . Volumetric proportions of testicular element

The volumetric proportions of the various testicular elements are shown in Table 2. The three different treatments showed no significant difference in terms of the relative proportions of the testis occupied by the various elements viz: secondary spermatocyte, round spermatid (R^2), round spermatid (R^3), zygotene 1^0 spermatocyte, elongating spermatid (el_4), elongating spermatids (el_5 – el_{10}), cytoplasm and seminiferous tubules.

Sertoli cell was significant (P<0.05) when FF treated group was compared with the control. Similarly, the lumen was significant (P<0.05) when TF treated group was compared with the control. Control group had the highest mean volume percent of pachytene 1^0 spermatocyte when compared with the treated groups and this was significant (P<0.05) when mean volume percent of pachytene 1^0 spermatocyte in control group (5.215) were compared with the FF treated group (3.921) and this represents on the average 33% above FF treated group, Table 2. Control group also had the highest mean volume % of diplotene 1^0 spermatocyte when compared with the treated groups and this was significant (P<0.05) when mean volume percent of diplotene 1^0 spermatocyte in control group (4.710) were compared with the FF treated group (3.576), Table 2. The most frequently occurring gametogenic elements were the primary spermatocytes at the pre-leptotene stage of meiotic prophase with 9.644%, 11.997% and 20.405% for both control, TF and FF treated groups respectively. The pre-leptotene 1^0 spermatocyte was not significant between the treated groups but the pre-leptotene 1^0 spermatocyte was significant (P<0.05) when TF treated group was compared with the control. The FF treated group was not significant in pre-leptotene spermatocyte when compared with the control groups (Table 2). The overall volume percent of spermaogonia, primary spermatocytes, round spermatids and elongating spermatids were 16.484, 28.949, 10.615, 10.660 for control groups, 10.034, 29.860, 10.182, 15.093, for TF treated groups, and 17.418, 26.2244, 11.052, 13.472 for FF treated groups respectively. The most transient elements encountered were elongating spermatids (el_4) trying to complete their elongating with some spermatids being located between the lumen and the basement membrane (2.093%, 2.037%, 2.190%). Other transient elements include the lumen (1.424, 2.469, and 2.000) in control, TF and FF treated groups respectively. There were significant differences in the volume percent of round spermatids (R_1) in the three different groups (Table 2) with control having the highest volume percent (3.297). There is a significant difference in volume percent of spermatozoa in control groups (5.142) as compared to the TF and FF treated groups (3.759 and 2.914) respectively.

3.4. Diameter and volume of spermatogenic elements

The mean tubular diameters for the control, TF and FF treated groups irrespective of the stage of the cycle of the seminiferous epithelium were 203.46, 195.42 and 197.13 microns, respectively and were highly significantly

influenced by the various hormonal fluids or treatments (Table 1). The mean tubular diameter of the control group is significantly more than the rest (as above) averaging 3.21% and 4.12% above mean tubular diameter in TF and FF treated groups respectively.

Table 2
Volumetric Properties of Testicular Elements in three different Treatments.

	Normal saline (control)		Testicular Fluid (TF)		Follicular Fluid (FF)	
Spermatogonia A	7.953^{a}		4.928^{b}	10.034	8.496^{a}	
Spermatogonia B	8.53^{a}	16.484	5.106^{b}		8.922	17.418
Preleptotene 1^0 Spermatocyte	9.644^{b}		11.997^{a}		10.405^{ab}	
Leptotene 1^0 spermatocyte	5.684^{a}		6.512^{a}		4.652^{b}	
Zygotene 1^0 spermatocyte	3.696^{a}	28.949	3.151^{a}	29.860	3.395^{a}	26.244
Pachytene 1^0 spermatocyte	5.125^{a}		4.624^{ab}		3.921^{b}	
Diplotene 1^0 spermatocyte	4.710^{a}		3.576^{b}		3.871^{ab}	
Secondary spermatocyte	10.402^{a}		11.220^{a}		8.831^{a}	
Round spermatid R_1	3.297^{a}		2.903^{b}		2.6847^{b}	
Round spermatid R_2	3.618^{a}	10.615	3.424^{a}	10.182	4.244^{a}	11.052
Round spermatid R_3	3.700^{a}		3.855^{a}		4.124^{a}	
Elongating spermatid (el_4)	2.093^{a}	10.660	2.037^{a}	15.093	2.190^{a}	13.472
Elongating spermatid (el_5 – el_{10})	8.567^{a}		13.056^{a}		11.282^{a}	
Spermatozoa	5.142^{b}		2.9144^{a}		3.759^{c}	
Sertoli cell	3.905^{a}		3.372^{ab}		2.985^{b}	
Lumen	1.424^{b}		2.469^{a}		2.000^{ab}	
Cytoplasm	4.034^{a}		4.412^{a}		4.519^{a}	
Intertubular space	2.294^{b}		3.198^{a}		2.760^{c}	
Seminiferous tubule	6.091^{a}		7.245^{a}		6.959^{a}	

^{a,b,c}Means in the same row with different superscripts are significantly different (P<0.05).

Table 3
Stages of the Cycle of Seminiferous Epithelium Frequencies (%) in Three Different Treatments.

Stages	Normal saline (control)	Testicular fluid	Follicular fluid
1	27.6^{a}	25.4^{b}	24.6^{b}
2	13.0^{a}	13.8^{a}	13.8^{a}
3	7.6^{a}	14.0^{b}	13.2^{b}
4	11.0^{a}	6.8^{b}	6.2^{b}
5	4.2^{a}	6.2^{b}	7.4^{b}
6	15.8^{a}	17.4^{b}	16.4^{ab}
7	12.2^{a}	12.0^{a}	11.8^{a}
8	8.6^{a}	4.4^{c}	6.6^{b}

^{abc}Means in the same row with different superscripts are significantly different (P<0.05).

3.5. Relative frequency and duration of the stages of the cycle of seminiferous epithelium

The relative frequencies of occurrence and duration of the stages of the cycle of seminiferous epithelium are presented in Table 3. The most frequently occurring stage was stage 1 in the three different groups followed by stage 6 while stage 5 remained the least frequent in control group, stage 8 remained the least in TF treated groups and stage 4 and 8 the least frequent in FF treated group (Table 3). There were significant differences in the relative frequencies and durations of the stages of the cycle of seminiferous epithelium of control group when compare

with the treated groups in stages 1, 5 and 8. In the stage 8 of the frequency duration of the cycle of seminiferous epithelium, there were significant differences among treated groups. The control group had the highest frequencies in the stages 1 and 8 of the cycle of seminiferous epithelium while the FF treated group had the highest frequency in stage 5.

4. Discussion

4.1. Body weights

Figures obtained pertaining to the weights showed that they grew significantly over their initial weights. Animal treated with testicular fluid showed highly significant weight increase ($P<0.01$) of 0.78kg, which on the average represents 110.8% and 95% higher in weight gain than those of follicular and control group respectively. The weight gain on testicular fluid treated group was twice as superior to the other two groups showing that the testicular fluid is a growth stimulant.

4.2. Tubular diameter

The administration of inhibin from bovine follicular and testicular fluid showed a highly significant decrease ($P<0.01$) in the diameter of seminiferous tubules when compared with the control groups. This agrees with the work of Sarvamangala and Sheth (1984) that administration of inhibin from ram testicular and follicular fluids brings about a reduction in Follicle Stimulating Hormone (FSH) which leads to a reduction in tubular diameter. Thus, the difference between treated and control groups can be appreciated.

4.3. Cellular element

Administration of inhibin from bovine follicular fluid and testicular fluid showed an increase in size of lumen and a significant decrease in percentage number of spermatozoa. It could be explained that the treatment suppressed FSH secretion, which in turn reduced the percentage number of spermatozoa. This agrees with the findings of Steinberger (1972); and Duckett (1975) who suggested that no hormones were required for spermatogonial steps up to those of late pachytene and that FSH would be necessary only at the final stages of the cycle. There was a decrease in pachytene 1^0 spermatocyte in the treated groups and this is in agreement with the work carried out by de Jong *et al* (1978) who observed a decrease in pachytene 1^0 spermatocyte when immature rats were treated with follicular fluid for 12 days. Thus the effect of inhibin on spermatogenesis appears to be via FSH secretion.

4.4. Cycle of seminiferous epithelium

Figures obtained pertaining to the stages of the cycle of seminiferous epithelium show that there were significant differences ($P<0.01$) in stages 1-6 in control groups when compared with treated groups. But there was no statistical significance among the treated groups (Table 3). The stages in the cycle of the seminiferous epithelium followed a continuous flow pattern among the treated groups (Table 3). There was a conspicuous increase in the occurrence of spermatozoa in stage 8 as compared to the treated groups, which suggest attainment of puberty. The control group values obtained in all the stages in this experiment correspond to an extent with values obtained by Swierstra and Foote (1963); which showed that the control groups were actively matured within 20 weeks old. Characteristic features of the stages of the cycle seen in the treated groups seem to be unique to the group because cellular association is not well established in the immature male (Gondos *et al*, 1973). In the light of these differences in the relative occurrence of each stage, it is further shown that the duration of spermatogenesis and of the stages of the cycle of the seminiferous epithelium is treatment specific.

4.5. Daily sperm production and daily sperm production per gram testis

The penultimate parameter used in this study was the determination of daily sperm production by quantitative histology based on the observation that this parameter could be determined at any point of spermatogenesis (Swierstra, 1966).

Daily sperm production was higher in control group than treated groups although not significant. Between the two treatments, the follicular fluid seems to show a greater impact on daily sperm production. Daily sperm production per gram testis follows the same trend. Swierstra (1966) observed that within species, sperm

production is a function of testicular size, since there was no testicular difference in weight observed in the treated and control groups while round spermatid counts were similar, it follows that this is a necessary observation. Pauffer and Foote (1969) and Hafez (1970) shared this view.

5. Conclusion

Administration of inhibin from bovine testicular and follicular fluids to pre-pubertal rabbits decreased the weights of pituitary gland, which implies that the administration of inhibin to immature rabbits affects the hypothalamo-pituitary function as well as block FSH dependent steps in spermatogenesis. There was a highly significant spermatozoa decrease ($P<0.01$) in the follicular fluid treated groups when compared with control group and this supports the ability of the inhibin from bovine follicular fluid to inhibit the onset of active spermatogenesis. Thus, the effect of inhibin on spermatogenesis appears to be via FSH secretion. The inhibin from charcoal - extracted bovine follicular and testicular fluids reduced the circulating concentrations of testosterone and FSH, thus allowing the evaluation of the role testosterone and FSH plays in testicular function. The depressed testicular activities (spermatozoa concentration) following the treatments support the hypothesis that in buck, spermatozoa development is attributed to circulating concentrations of testosterone and FSH.

The administration of follicular and testicular fluids and the resulting suppression of spermatozoa significantly affect concentrations of circulating testosterone. It is recommended that further work be carried out in which the treated buck are allowed to mate the doe to determine the beneficial effect of delayed puberty on embryo survival, litter size and survival of the kindle.

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