IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.301
Volume 3, Issue 3, March 2023

# Evaluation of New Zealand White Rabbit Semen Raised in the Coastal Region

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***Abstract:*** *For successful rabbit, rearing one should know the basics of rabbit reproduction. One single male can affect the fertility and prolificacy of about one hundred females when A. I. is performed. Elevated temperature and humidity increase the testicular temperature which ultimately decreases the semen quality, therefore present study was conducted to determine different semen characteristics to predict fertility of rabbit semen raised in coastal region. Four male rabbits of New Zealand White breed were selected. Six ejaculates were obtained from each rabbit by artificial vagina method. Macroscopic and microscopic semen parameters were evaluated. The average volume Colour and Consistency, Density, pH were 1.23 ± 0.10 ml, Milky White, Thick to Thin, DD(D) to DDD and 7.5 ± 0.05 respectively, whereas Mass Activity, Initial Motility, Live sperm percentage, Abnormal sperm percentage, Total sperm concentration were ++ to ++++, 75.42 ± 1.04 %; 87.96 ± 0.41 % ; 11.54 ± 0.87 % and 291.50 ± 4.05 x 106 /ml. The average hypo osmotic swelling test percentages were 79.79±0.84% and whereas average acrosome intact percentages were 85.25 ± 0.54%. All the semen parameters were within the normal range, which indicate good fertility of semen. Hence it can be concluded that these four male rabbits of New Zealand White breed can be utilized for AI in does/ female rabbits.*

***Keywords:*** Zealand White Breed, Semen Characteristic, Artificial Vagina, Temperature Humidity Index.

## I. INTRODUCTION

Proper selection of breeding male is prerequisite and important aspect in rabbit breeding development program because a buck can produce thousands of kids a year through artificial insemination (AI). Therefore, for better propagation and genetic improvement of rabbit species there should have good breeding buck.

Increase in temperature with high temperature causes heat stress in livestock and causes negative impacts on livestock is a major challenge in subtropical regions. sweat glands in rabbits are in limited number to lose excess produced heat. Thus, the heat stressed rabbits have high rectal temperature. A temperature of 13-20° C is known as the comfort zone for rabbits but at higher temperature, the appetite depressed, the productive and reproductive performances are impaired and the resistance to disease is decreased (Marai et al., 2002 and 2008). In coastal region the temperature is ranged from 25 to 350C with 75 to 100 % humidity. In coastal area the breeds of livestock showed some dissimilarity from other livestocks raised in other regions. Paul, et al. (2021)

The macro and microscopic characteristics of semen plays important role in selection of breeding male stock. It also gives the idea of good semen quality and helps in early detection of impaired fertility in males due to poor quality of semen. Literature revealed certain studies on the seminal attributes of some of the rabbit breeds e. g. Californian, New Zealand White, Sinai and Balady etc. however, information on rabbit buck raised in coastal region was scanty in New Zealand White breed. So, the study on the semen characteristics of New Zealand White buck was carried out.

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Volume 3, Issue 3, March 2023

## II.MATERIALS AND METHODS

Present study was carried out from April 2011 to June 2011 in Department of Animal Reproduction, Gynaecology and Obstetrics, at Mumbai Veterinary College, Mumbai-12 and ICAR Research Complex for Goa, Ela, Old Goa. Four clinically healthy and sexually matured male rabbits of New Zealand White breed kept at ICAR - Central Coastal Agricultural Research Institute, Ela, Old Goa - 403 402. were selected for the present study.

1. **Management of Bucks:** All rabbits were clinically healthy and showing normal sexual behavior. They were housed in individual cages. These rabbits were maintained under same standard feeding and managemental practices. They were supplied with green grass 300 gm, concentrated pellet feed 80 g and ad lib clean drinking water daily. They were practiced mounting on doe at every two days interval.
2. **Temperature humidity index (THI) estimation:** Air temperature (°C) and relative humidity (%) inside the rabbitry building were measured once a day weekly 3 times study period. The temperature-humidity index (THI) was calculated using the equation as follows:
   THI = db°C – [(0.31-0.31 RH) (db°C -14.4)]
   (Wheredb°C=dry bulb temperature in Celsius and RH= relative humidity percentage /100).
   The THI values obtained were then classified as follow: <27.8= absence of heat stress, 27.8 to < 28.9= moderate heat stress, 28.9 to <30.0 = severe heat stress and 30.0 and more = very severe heat stress (Marai et al., 2002).
3. **Semen Collection:** The semen was collected from each rabbit by artificial vagina method as described by Chakurkar et al. (1996). The artificial vagina was designed in the laboratory and it consisted of a soft rubber tube directly connected with a graduated conical glass tube. The temperature of artificial vagina was adjusted to 42 to 450C at the time of semen collection. All the ejaculates were collected early morning between 6.00 to 6.30 am on empty stomach to avoid contamination of semen sample by feed or faeces. The prepucial sheath was washed with normal saline before semen collection. At semen collection the bucks were allowed one false mount and at the subsequent mounting the artificial vagina was adequately positioned for penis intromission. Bucks have been previously adapted to this routine and no refusal occurred. All ejaculates were stored at 37ºC in a water bath until further semen evaluation.
4. **Semen Evaluation:**The routine evaluation of fresh semen was performed as described by Sane et. al. (1994).
   **Macroscopic test- these tests are as follows:**
   I. **Volume:** Immediately after semen collection the gel fraction is removed by forcep and then the volume of fresh semen was recorded from the graduated semen collecting tube.
   II. **Colour and Consistency:** The colour and consistency of the semen was recorded in the collecting tube immediately after collection by naked eyes. Semen with a watery consistency was readily distinguished from that with a viscid consistency. The colour of rabbit semen was creamy white to milky white. Any deviation from the normal colour was considered abnormal. The consistency of semen gives a rough indication of the concentration of spermatozoa. The neat semen sample was held against a white background and the colour and consistency were and is given in Table 1.
   III. **Density:** It gives a rough estimation of the percentage of spermatozoa in the semen sample. The neat semen sample was held against natural sunlight and the density was graded as D, D (D), DD, DD (D), DDD, and DDD.
   IV. **pH (Hydrogen ion concentration)**: The pH was measured with the help of pH paper. A strip of paper was dipped into freshly collected semen in the graduated tube. The change in colour of pH paper was then compared with the colour of the pH paper strip/indicator book. The corresponding figure indicates the pH of the semen.

**Table 1 Grading of semen colour and consistency**

| Sr. No. | Colour | Consistency |
|---|---|---|
| 1 | Creamy | Thick<br>Thin |
| 2 | Milky | Thick<br>Thin |

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| | | |
|---|---|---|
| 3 | Cloudy | Thick<br>Thin |
| 4 | Watery | Thick<br>Thin |

**Microscopic Tests:** following tests were conducted and detail test procedure is as follows:

I. **Mass Activity:** Mass activity of the semen sample was examined immediately after semen collection. The collection tube containing the semen sample was closed with an aluminum foil or cotton plug and was kept in a water bath at around 37° C temperature. A drop of semen was placed on a clean pre warmed slide and observed under the low power without applying a cover slip. The semen samples showed a series of rapidly changing swirls, waves, and current of sperm motion. On the basis of the mass movement, the semen was graded into the following categories (Table.2).

**Table2 Grading of mass activity of neat semen.**

| Sr. No. | Motion of sperms | Grade |
|---|---|---|
| 1 | Nil motion | 0 |
| 2 | Movement slightly vigorous, but no eddies or current formed. | + |
| 3 | Waves with slight whirls which move across the field. | ++ |
| 4 | Rapid and vigorous waves with swirls or eddies which change with rapidity. | +++ |
| 5 | Extreme churning swirls and eddies. | ++++ |

II. **Motility:** The initial motility and motility of stored semen was estimated to observe semen quality. A drop of diluted semen was taken on a clean prewarmed glass slide at 30-35°C and seen under low power and high-power microscope using cover slip. Initial motility was graded as described by Sane et al. (1994) is given in Table 3.

**Table.3: grading of mass activity of neat semen**

| Sr. No. | Movement of sperm | Motile sperms ( %) |
|---|---|---|
| 1 | Nil | 0 |
| 2 | Poor motility (mostly weak and oscillatory) | 20 |
| 3 | Fair motility (sufficient motile, sperms with rapid, vigorous motion) | 40 |
| 4 | Good motility (very rapid, vigorous and progressive motion) | 60 |
| 5 | Very good (most vigorous with progressive swirling activity) | 80 |
| 6 | Excellent (highly vigorous progressive waves in all directions) | 100 |

III. **Total Sperm Concentration:** Estimation of total sperm concentration was done by using a haemocytometer. Diluting fluid was prepared by mixing Sodium citrate dehydrate (3gm), Eosin yellow (100mg) in 100mil distilled water. To kill the spermatozoa 0.5 ml Formalin was added to this solution.

**Steps for dilution of semen for total concentration-**

1. Dilution 1 in 1000- 0.1 ml of semen was diluted in 9.9 ml of NSS. Hence dilution was 1 in 100. 1 ml of the above was diluted in 9 ml of dilutor so that the dilution will be (1:1000).

2. Charging of chamber – A cover slip was gently placed fixed on the edge of the haemocytometer and a drop of the diluted semen (1:1000) was put at the edge of the cover slip with the help of a micro pipette.

3. Counting of spermatozoa- Spermatozoa were counted in the 16 small squares of all four large squares (i.e. 64 small squares).4. Calculation-N = Average no. of sperms counted in 4 small squares, 0.1mm3 contains N spermatozoa, 1mm 3 contains 10N spermatozoa. 1ml contains 10N x 1000 spermatozoa (1ml = 1 cm3), This

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was diluted semen (1: 1000)Therefore, 1ml of neat semen contains - 10N x 1000 x 1000 spermatozoa. The final calculation was as below.
Total concentration = 10N million sperms /ml = (10N x 106) ml

IV. **Live and Dead spermatozoa percentage:**The ingredients (Eosin yellow 1.67gm and Nigrosin 10 g ) were dissolved in distilled water to make it 100ml and the pH was adjusted from 6.8 to 7. A drop of semen was taken on a watch glass and to it 3-4 drops of Eosin-Nigrosin Stain was added and kept for 2 minutes. A smear was prepared and air dried. Dead spermatozoa took Eosin stain and appeared pink while live spermatozoa appeared white. partially stained or more than half stained spermatozoa were counted as dead. Total 333 spermatozoa were counted. Therefore, percentage of dead spermatozoa = No of dead spermatozoa × 3/10.

V. **Abnormal Spermatozoa Percentage:**Eosin–Nigrosin combined stain was prepared as described in live and dead sperm counting and used for counting the abnormal sperms. Classification of abnormal sperms was done as per Lagerlof's Classification (1944)Maximum 20% total abnormalities are permissible for a normal good quality semen. Permissible abnormalities were Head-10%, Mid piece-8% and Tail-2%.

VI. **Hypo Osmotic Swelling Test (HOS Test):**The hypo osmotic test was carried out as described by Bhosrekar (2005).Two hypo osmotic solutions were prepared 2.7% aqueous solution of fructose (1.351 gm) and 1.47 % aqueous solution of sodium citrate (0.735 gm). About equal volume of both solutions (0.5 ml each) were mixed and kept in an incubator at 37 °C for 10 minutes. In the above hypo osmotic solution 0.1 ml of semen was added and incubated at 37 °C for 30 minutes.10 µl of this mixture was taken on a glass slide and a cover slip was placed on it. The slide was observed under 40x objectives to determine percentage of swollen sperm heads and of coiled tails. Alternatively, 10 µl of this mixture was placed on a glass slide and a thin smear made. Air dry the smear and stain with Giemsa's staining solution for 45 minutes. Wash the slide with distilled water, air dry ad observes under 40x objective.

VII. **Acrosome Intact Test of Spermatozoa:** Acrosome damage was studied as per Hancock (1951). The ingredients were mixed thoroughly and filtered. The filtrate was then used as a "stock solution." Working staining solution was prepared by mixing 3ml of stock solution with 2 ml solution saline buffer at pH 6.8 and 45ml carbon dioxide free (i.e., double distilled) water. A thin smear of semen was made on a clean glass slide. This smear was then air dried and kept in 5 per cent formaldehyde solution for 30 minutes at 37° C temperature. The slide was washed in distilled water and air dried. The air-dried slides were then kept in a staining jar containing working solution for 3 h at 37°C. The stained slides were then washed with distilled water and air dried. Then 100 spermatozoa from each slide were counted under oil immersion and sperms with intact acrosome were noted.

VIII. **Statistical Analysis:** The recorded data were entered in the Microsoft Excel sheet. The average semen volume, Live sperm percentages, concentration of sperm, acrosome intact percentages and Hypo – osmotic swelling test (HOS test) were calculated expressed as mean ± SD. The individual sperm motility was expressed as the average percentage. The descriptive analysis and Correlations coefficients were calculated by Microsoft excel -2021 statistical program The analysis of variance (AVOVA) was done for test the significance. In case of all parameters, a statistical significance was considered at $p \leqslant 0.05$.

## III.RESULTS

From 4 buck total 48 collections were collected and data is presented in Mean ± SE. The collected weather data, showed the average 29 0C temperature (max 35& min 23) and average relative humidity (RH) was 77.77% with range from 51-95% during April, 2011 to June, 2011. The estimated THI values were 77.33% indicating the bucks were exposed to moderate heat stress during the study.

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Volume 3, Issue 3, March 2023

The averages of the pooled samples of macroscopic semen characteristics are given in Table 1. The average volume, Colour (Fig.1) and Consistency, Density, pH were 0.95 ± 0.06 ml, thick to thin, DD(D) to DDD and 7.5 ± 0.00 respectively. The average microscopic evaluation of semen characteristics is given in table 2, it was observed that the Mass Activity, Initial Motility, Live and Dead percentage, Abnormal sperm percentage (fig. 2), Total concentration were +++ to ++++; 69.58 ± 1.65 %, 14.25 ± 0.48 %, 10.79 ± 0.74 % and 315.40 ± 0.35 x 106 /ml respectively. The average HOST percentages were 76.58% with range from 66 to 83 % whereas average acrosome intact percentages were 82.17% with range 70 to 89 %.

All the bucks showed significant difference ($p < 0.05$) for live sperm percentages and total sperm concentration other semen parameters were non-significant (Table 5). There was positive correlation ($r= 0.50$) between semen pH and acrosome intact percentages. Similarly positive correlation ($r= 0.50$) between initial motility with acrosome also found. The Concentration of sperm per ml showed positive correlations ($r = 0.33$) whereas positive correlation coefficient ($r = 0.55$) between live sperm motility and HOS test was found. (Table 7).

## IV.DISCUSSION

The ejaculate volume ranges from 0.1 to 3.0 ml in mature rabbits (Fig.1) recorded by Demirci (1994).The individual (buck) selected for semen collection showed variation in the ejaculate volume but the overall average volume 1.23 ± 0.10 ml (range 0.6 ml to 2.4ml) of ejaculate observed in present study was higher than that recorded by Uysal et al. (2010), Rodriguez – De Lara et al. ( 2010 ) and Hassanien and Baiomy (2011) .The four bucks belonged to the same breed and of similar age their management and nutritional status and general health condition were also similar. So, the difference in the volume of semen might reflect their different genetic potentiality and genetically superior bucks could produce higher volume of semen El-Tarabany et. al. (2015). The higher volume noted by in the present study could be due to exposure of the bucks to does during semen collection as doe exposure act as a bio stimulation that increases in semen ejaculate volume, Rodriguez-De Lara et. al. (2010).

Rabbit semen is normally creamy white or milky white in colour but the best quality is found in creamy-white semen (Mataveli, 2008). Overall colour of neat semen in New Zealand White was milky white.Similar results were reported by Salcedo-Baca et. al. (2004), and Roychoudhury et. al. (2009). Whereas, Oyeyemi, &Okediran (2007) recorded the colour of the ejaculates were creamy white upon feeding of soyabean meal but became creamy as the percentage of soymeal increased. The presence of particles, non liquified streaks of mucus or debris requires further evaluation. Contamination of semen with urine gives yellow colour due to low temperature of Artificial vagina. Alvarez et. al. (2006) reported that the white colour of rabbit semen is depend on the sperm concentration.Throughout this study, which focused on the characterization of semen parameters of New Zealand White bucks, no abnormalities in semen color were observed in the buck.

The consistency of the seminal fluid may vary considerably both between different animals and between different semen samples from the same animal. A sample with thick consistency indicates a sample with good sperm concentration similarly in present study thick consistency of semen was seen in 19 collections and thin consistency in 5 collections was seen. The overall consistency of neat semen in New Zealand White was thick to thin. Dubiel, (1975) noted that the colour and consistency of the semen depend mainly on the concentration of the spermatozoa and lipids in the volume unit of the ejaculate.

Density of neat semen is an important part of semen analysis it reflects the total concentration of the spermatozoa in a sample. Samples with higher density have an increased total sperm concentration. Present study showed that, DD (D) density was seen in 6 bucks and DDD density was seen in 18 bucks. The overall density of neat semen of New Zealand White Rabbits ranged from DD(D) to DDD indicating having a good concentration of semen.

The pH should only be recorded of normal fresh semen as it may slowly fall as the sample ages. The average normal pH of rabbit semen is 7.5. Inflammatory conditions particularly of the prostrate and seminal vesicles may alter the pH of semen. In addition, alteration in the pH of semen can be due to faecal and urine contamination. It was observed that the average pH noted for neat semen of New Zealand White buck was 7.58 ± 0.05. Semen pH was negatively correlated with concentration, mass motility and the percentage of motile spermatozoa. (Garcia – Tomas et. al. 2006b and El-Tarabany et al., 2015). They explained as this was due to the metabolic activity of the spermatozoa which releases lactic acid and causes a reduction in the pH.

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**Table 4. Macroscopic Characteristics of New Zealand White**

| Buck No | Volume | pH | Colour | Consistency | Density |
|---|---|---|---|---|---|
| C13 | 1.65 ± 0.33 | 7.50 ±0.00 | Milky White | Thick | DD(D) |
| C14 | 1.18 ±0.09 | 7.82 ±0.20 | Milky White | Thick | DDD |
| C15 | 1.12 ±0.13 | 7.50 ±0.00 | Milky White | Thin | DDD |
| C16 | 0.95 ±0.08 | 7.50 ±0.00 | Milky White | Thin | DD(D) |
| Total | 1.23 ± 0.10 | 7.58 ± 0.05 | Milky White | Thick | DDD |
| Significance | NS | NS | | | |

**Table 5. Microscopic semen parameter of New Zealand White**

| Buck No | Mass Motility | Initial Motility | Live | Abnormal % | | | | Concentration |
|---|---|---|---|---|---|---|---|---|
| | | | | Head | Mid Piece | Tail | Total | |
| C13 | +++ | 73.33 ± 2.11 | 86.00 ± 0.73$^{b}$ | 3.83 ±0.60 | 1.83 ±0.60 | 5.00 ±0.45 | 10.67±1.26 | 282.50 ±5.12$^{a}$ |
| C14 | +++ | 75.00 ±2.24 | 88.83 ±0.98$^{a}$ | 6.33± 0.95 | 2.50 ±0.43 | 5.50 ±1.54 | 14.33± 2.72 | 279.00 ±10.23$^{b}$ |
| C15 | ++++ | 76.67 ±2.11 | 87.83 ±0.48$^{ab}$ | 4.17± 0.48 | 1.83 ±0.87 | 4.67 ±0.56 | 10.67± 1.38 | 296.67 ±6.15$^{ab}$ |
| C16 | ++++ | 76.67 ±2.11 | 89.17 ±0.31$^{a}$ | 4.00±0.86 | 1.00 ±0.37 | 5.50 ±1.06 | 10.50± 0.76 | 307.83 ±4.87a |
| **Total** | +++++ | **75.42±1.04** | **87.96 ±0.41** | **4.58 ±0.41** | **1.79 ±0.31** | **5.17 ±0.45** | **11.54 ±0.87** | **291.50 ± 4.04** |
| Significance | | NS | * | NS | NS | NS | NS | * |

a, b: values assigned by different letters on the same column are significantly different ($p < 0.05$);

**Microscopic Evaluation of semen characteristics:**

Motility or movement of spermatozoa is an index of their activity and it represents the quality of the semen. Table 2 showed, that the average mass activity of neat semen recorded in the present research work in New Zealand White rabbit was with range from +++ to ++++. are comparable with those recorded by Echegaray – Torres (2004) and Garcia –Tomas et al. (2006a) who observed mass motility in New Zealand White rabbit buck semen was 2.70 on a scale from 1 to 3 which was equivalent to ++++. This indicate that spermatozoa having more energy (fructose) will move within the medium rapidly and for a long time.

It is known that only progressively motile sperm cells have fertilising ability. table no 2. showed that the initial motility in the present study for New Zealand White was 75.42 ± 1.04 % which is with in normal range, which is in close agreement with Safaa et al. (2008), whereas Panella et al. (1993), Uysal et al. (2010) and Hassanien and Baiomy (2011) recorded lower motility percentages than present observations. The present observations showed an acceptable motility percentage and this motility illustrates the degree of sperm activity and its importance for passing during the oviduct and completing fertilization (El-Darawany and El-Sayiad, 1994 and Jean et al., 2002).

Determining of total dead spermatozoa percentage is necessary in order for selection of animals for breeding programmes. Rabbit semen of good quality should not possess more than 25 % of dead sperm Ax et al. (2000). If the percentage of dead sperm exceeds 25 % then fertility decreases dramatically Uysal et al. (2010). Table 2. showed that the total dead and Live sperm percentage in the present study for New Zealand White were 12.04 ± 0.41 and 87.96 ± 0.41% respectively. Salcedo–Baca (2004) and Rodriguez –De Lara et al. (2010) reported similar dead percentages, while Uysal et al. (2010) recorded higher values. Although the semen parameters are altered by multiple factors.

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Rodríguez-De Lara et al. (2010) reported that live sperm percentages decrease as the weight of the bucks increases. However, in present study live and dead percentages are within normal range for good fertility.

Every semen sample contains abnormal sperm cells. The many structural abnormalities that may occur in a sperm are known to be associated with infertility. Average head, mid piece and tail abnormality were 4.58 ± 0.41, 1.79 ± 0.31 and 5.17 ± 0.45%. Marco – Jimenez et al. (2005) reported that bucks with sperms having small head size showed lower fertility (45 per cent) then bucks with sperms having larger head size (77.90 per cent). Daader et al. (1997) recorded that sperm abnormalities were higher in summer. Demonstrations of their presence in a semen sample are of great importance in determining the potential fertility of that semen. When abnormal sperm cells exceed 25.00 % fertility typically declines.

In the present study the average sperm abnormality percentage recorded was 11.54 ± 0.87 %. The present observation was higher than those recorded by higher than those recorded by Salcedo–Baca et al. (2004) and Rodriguez–De Lara et al. (2010). The total abnormal sperm percentage was within the normal limit i.e., below 20%. This finding was in agreement with Uysal et al. (2010) who reported that the morphological sperm abnormalities (20.00 ± 0.52 per cent) in buck semen did not reach to the values which affect the fertility for each breed and individual. Correa et al. (1997) observed that sperm samples with high incidence of abnormal sperm (24.10%) had a lower fertility than those samples with a low percentage of sperm abnormalities (17.80%). They opined that in polytocous species, the high incidence of abnormal sperm in a sample not only results in a decreased fertility rate, but prolificacy is also affected.

The sperm concentration describes the number of sperms in million that are present per unit volume of seminal fluid. The total sperm per ejaculate is an important semen variable related to fertility, more concentrated semen is characterized by a higher value of live sperms, progressive motility, and fertility. Table 2, indicate the average total sperm was 291.50 ± 4.04 x 106 /ml. present finding in close agreement with that reported by Rodriguez–De Lara et al. (2010) but lower than that recorded by Panella et al. (1993), Carluccio et al. (2003), Salcedo–Baca et al. (2004) Hassanien and Baiomy (2011). Variation in total sperm concentration could be due to individual variation, age, nutrition, environment, and health status of animal. Panella et al. (1993) reported that different estimation method (photometer vs. Thoma chamber) and collection frequency can cause variation in the total sperm concentration. In the present study the total sperm concentration was estimated by hemocytometer and semen was collected twice a week. The number of sperm produce in the present study indicates higher the capacity of the testicular cells for spermatogenesis and therefore more number of sperm produced. Similar observation was recorded by Oyeyemi and Okediran (2007).

Among sperm function test the study of plasma lemma is of particular importance since a biochemically active membrane is required in the process of capacitation, the acrosome reaction and the binding of the spermatozoa to the oocyte surface Correa and Zavos (1994). The membrane function test i. e. hypoosmotic swelling test (HOS test) and Acrosome intact test yields information regarding sperm membrane functionality. The average HOS test percentage in New Zealand White rabbit was 76.58 %. Jeyendran et al. (1984) reported that spermatozoa showing swelling of entire tail region accounted for maximum amount of the total swelling recorded for the HOST test. This was in agreement with the results obtained in the present study.

The process by which the enzymes are releases is the result of fusion and vesiculation of the plasma membrane specifically covering the acrosome and the outer acrosomal membrane. This process has been termed the acrosome reaction and occurs universally in mammalian spermatozoa. From table 2, it was observed that the average acrosome intact percentage in the present study for New Zealand White rabbit was 82.17%. The present findings for New Zealand White rabbit higher than those reported by Napier (1961), Since only sperm that maintain an intact acrosome can take part in fertilizing an oocyte the percentage of sperm with damaged acrosome should be low in order to maintain high fertility levels Lavara et al. (2005). The present findings for acrosome reaction percentages are within normal limits indicate the good fertility of semen.

Microenvironment is the immediate physical environment surrounding an animal; such as the cage, pen, or stall (Parker et. al., 2003). It is always helpful to know the microenvironment of our intensively reared livestock because it gives the general idea of our efficient management practices for livestock raising. Due to global warming, environmental temperature and relative humidity causes heat stress in livestock. Therefore THI is an indicator used to measure the effect of heat stress on ruminant production in tropical or subtropical areas (Bouraoui et al., 2002).

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The rabbit is comfortable and maintain thermoregulation between 18 to 21 °C. (Marai, Ayyat, and Abd El-Monem, 2001). In this study's region, THI had between (27.8 to < 28.9). according to (Marai et al., 2002) the New Zealand White bucks were experienced moderate stress during April, 2011 to June 2011. Rabbits do not have enough functional sweat glands to lose excess produced heat. Thus, the heat stressed rabbits have high rectal temperature (Jimoh and Ewuola, 2018). Gacek (2002) observed that high ambient temperature can impair the reproductive performance of rabbits, and 30 °C is considered as the threshold point beyond which infertility may result.

Severe heat stress adversely affects their hormonal and biological functions as well as mineral metabolism causing reduction in testosterone production and increase in cortisol level Habeeb et. al. (2018). Under heat stress there is less semen volume produce by buck, this may be due to hypo activity of the accessory glands and the testes and the lower sperm concentration is due to low testosterone concentration which affects spermatogenesis. It ultimately shows less mass activity and initial motility in rabbit semen during heat stress (Bonnano and Costanzo,1987). Rabbit spermatozoa display high metabolic activity and they are rich in polyunsaturated fatty acids in plasma membrane, which may cause increasing lipid peroxidation on exposure to heat stress. (Attia et. al. 2017), so they are sensitive to free radical's attacks. This may result in reducing sperm motility, fragmentation of DNA and reducing sperm fertilizing ability (Attia et. al. 2019). Also, an excessive free radical's production exceeds the antioxidant capacity of the seminal plasma, leading to damaged mitochondria and membranes (acrosomal and plasma) of spermatozoa (Mizera et. al. 2019).

The negative effects of heat stress conditions on ejaculate volume, sperm motility, number of motile sperm per ejaculate, sperm-cell concentration and total sperm-output of the buck rabbits were reported by several scientist (Marai et. al. 2002; Habeeb et. al., 2008; Theau-Clément et. al., 2015 and El-Rate et. al. 2021), however the semen parameters of New Zealand White breed are within the acceptable range under moderate heat stress on during the study period.

In conclusion, according to the results of this study showed even after exposure to the moderate heat stress the principle stemmatological parameters determined in the semen of New Zealand White breeds are within nearly normal limits suggesting no reproductive disorders found were among when they were evaluated for sperm parameters, therefore the semen of New Zealand White possess good quality in this study and suitable for preservation and Artificial Insemination.

**Table 6. sperm function test of New Zealand White**

| Buck No | Acrosome | HOS Test |
|---|---|---|
| C14 | 85.67 ± 1.45 | 80.33 ± 1.73 |
| C15 | 86.33 ± 0.95 | 81.83 ± 1.05 |
| C16 | 85.50 ± 0.81 | 80.67 ± 1.73 |
| **Total** | **85.25 ± 0.54** | **79.79 ± 0.84** |
| Significance | NS | NS |

None of the mean ± SE values among buck differed significantly (P>0.05)

**Table 7 Correlation between sperm parameters of New Zealand White bucks**

| Variables | Volume | Ph | Initial Motility % | Live % | Sperm Conc. | Acrosome % | HOS test % |
|---|---|---|---|---|---|---|---|
| **Volume** | 1.00 | | | | | | |
| **Ph** | 0.01 | 1.00 | | | | | |
| **Initial motility** | -0.28 | 0.28 | 1.00 | | | | |
| **Live** | -0.15 | 0.32 | 0.20 | 1.00 | | | |
| **Concentration** | -0.30 | -0.06 | 0.29 | 0.21 | 1.00 | | |
| **Acrosome** | -0.16 | 0.50 | 0.55 | 0.34 | 0.33 | 1.00 | |
| **Hos test** | -0.28 | 0.02 | 0.01 | 0.55 | 0.14 | 0.38 | 1 |

IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.301
Volume 3, Issue 3, March 2023

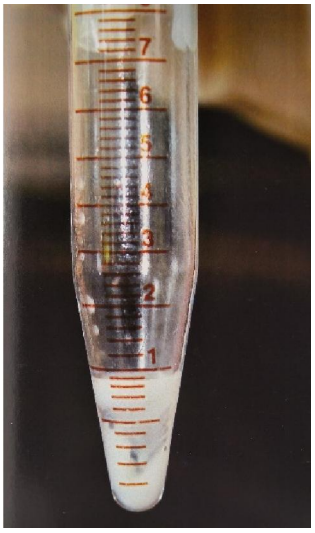


**Fig. 1. New Zealand White Buck Semen-milky white colour**

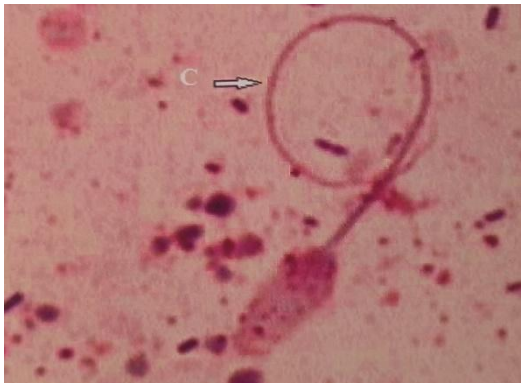


**Fig. 2.Abnormal Spermatozoa - coiled tail(C)**

## ACKNOWLEDGEMENT

Author would like to thank the Director, ICAR - Central Coastal Agricultural Research Institute, Ela, Old Goa - 403 402 and Principal Scientist, Dr. E. B. Chakurkar, for providing the necessary facilities to carry out the Masters's research.

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IJARSCT
ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
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Volume 3, Issue 3, March 2023

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ISSN (Online) 2581-9429
International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
Impact Factor: 7.301
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