



E-ISSN: 2320-7078

P-ISSN: 2349-6800

[www.entomoljournal.com](http://www.entomoljournal.com)

JEZS 2020; 8(3): 920-925

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Received: 04-03-2020

Accepted: 06-04-2020

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## Cellular exploration of pars distalis adenohypophysis in mature and immature male and female rabbit (*Oryctolagus cuniculus*)

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### Abstract

To study the cellular details of Pars distalis adenohypophysis of pituitary gland twelve healthy immature and mature rabbits were utilized. The pars distalis adenohypophysis comprised of cells arranged in irregular cords, grouped as acidophils, basophil and chromophobes. Type I Acidophils were usually round or oval and rarely elongated with distinct outlines its rounded nuclei were eccentrically placed. Type II acidophils were rounded, irregular polygonal and rarely elongated with densely granulated cytoplasm. The cytoplasm of Type I Basophil found densely granulated with coarse granules. Type II basophils were irregularly rounded, oval or angular with rounded nuclei and cytoplasm showed fine granules. The chromophobes were weakly stained and sparsely distributed throughout the adenohypophysis pars distalis. The size of the cells was observed that Type I acidophils were largest followed by Type II acidophil, Type I basophil and type II basophils. Sexual and age-related variations were not noticed.

**Keywords:** Histology; rabbit; age; sex; pars distalis adenohypophysis.

### Introduction

The hypophysis cerebri or pituitary gland is an endocrine gland specific to vertebrate. This gland integrates signals from the hypothalamus and periphery and plays central roles in regulation of vital processes involved in homeostasis, metabolism, growth and reproduction [3]. It sometime called the master gland of the endocrine system, because it controls the functions of the other endocrine glands. The adenohypophysis has three region the pars distalis or anterior lobe, the pars intermedia or intermediate lobe and the pars tuberalis. They synthesize and release protein hormones necessary for normal growth and development and also stimulate the activities of several target glands. Pars distalis has three principal cell types, chromophobes, acidophils and basophils, which secrete different hormones [11]. The cytological alteration may affect the increased or decreased production of such hormones. The present study was planned to explore the histological picture and cell types of adenohypophysis pars distalis of mature and immature rabbits of both sexes.

### Materials and methods

**Experimental animals:** A total of twenty-four rabbits in which twelve healthy immature (one month of age) and 12 healthy mature (4 month of age) rabbits were utilized in this investigation. Out of the immature and mature rabbits six were males and six were females. The rabbits were procured from the government of west Bengal, directorate of Animal resources, sheep, goat and rabbit breeding farm, Buddha park, Kalyani, Nadiya, which being reared for meat purposes. The rabbits were maintained in the department of Veterinary Anatomy and Histology, West Bengal University of Animal and Fishery Science, Kolkata-37, India. The experimental animals were grouped according to their age.

Group A (Immature male) - 1 month of age

Group B (Immature female) - 1 month of age

Group c (Immature male) - 4 month of age

Group A (Immature female) - 1 month of age

**Histological observations:** After scarifies of animals, open the cranial cavity and collect the

pituitary gland very carefully. Tissue samples were preserved in Bouins solution for 48 hrs for proper fixation. All collected tissues were processed by routine histological procedure and paraffin blocks were cut at 5-6  $\mu\text{m}$  thickness for histological study. The sections were stained with Mallory-Azan (Heidenhain's) method for endocrine cells in adenohypophysis [2].

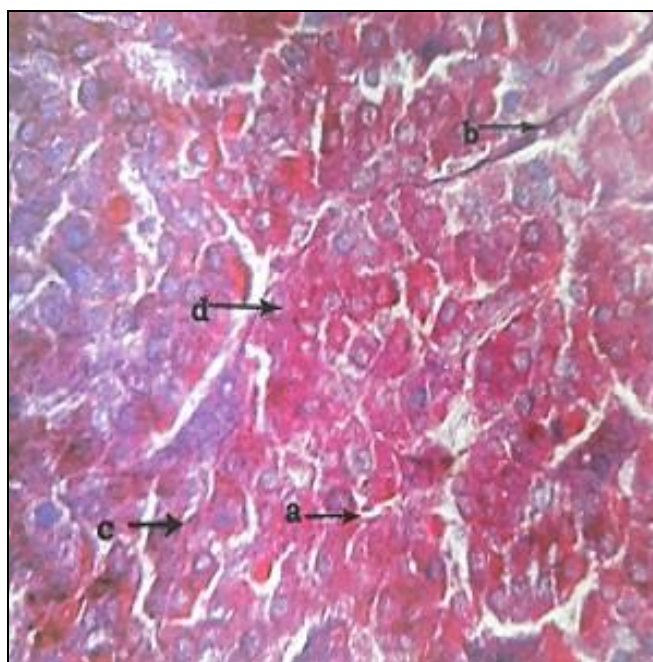
**Micrometrical observation:** Size of the cell and nucleus of different cell types was measured by ocular micrometer. At least 30 cells of each cell types were measured for their maximum and minimum diameter randomly in the different region.

**Statistical analysis:** The data was generated from histological findings by statistical analysis to one way ANOVA for various age groups [19].

### Results and discussion

The cells of adenohypophysis pars distalis were arranged in groups of variables size either in the form of cords or in the form of acini in mature rabbits. Sometimes, isolated cells

### Photograph



**Fig 1:** Photomicrograph of pituitary gland of mature female rabbit showing pars distalis. a- sinusoids, b- connective tissue septa, c- irregular cellular cords, d-lobules.

Azan X400

### Acidophils

The acidophils varied in shape from round, oval, polygonal, elongated or angular. They occurred mostly in groups of 2-3 cells and sometimes larger groups were also observed. Nuclei were mostly eccentric in position as findings of [13] in Red sheep. In mature males they constituted 45.27% of total cell count and the said value for mature female was 48.26%. In immature rabbits it was 44.11% and 43.76% for male and females respectively (Table 1). The acidophil cells constituted 35% of total cell count of pars distalis in human [10] and [6].

### Type I acidophil

These cells were usually round or oval and rarely elongated with distinct outlines. The nuclei were mostly round, rarely

were also found. The cells were found to be large and distinct. Connective tissue in between cells was prominent (Fig.1). In immature male rabbit cells were arranged in short irregular cords rather than in follicles and acini. Isolated cells were rarely found and the connective tissues between the cell cords were poorly developed. The parenchyma of adenohypophysis pars distalis of immature females exhibited similar histomorphological organization to that of immature male. These findings are in accordance with observation of [14] in sheep and [7] in human. However, in immature rabbits of both sexes the parenchyma containing short irregular cords were observed but no proper acini of follicles were detected. These findings were in accordance with [15], who recorded that pars distalis of fetuses of goat contained numerous anastomosing cellular cords and separated by sinusoids.

On primary observation under light microscope three types of cell that is Acidophils, Basophils and chromophobes were identified depending upon their staining characters in accordance with [1] in buffalo and [11] in rat. On the basis of the morphology of the cells and their arrangements within the parenchyma, the acidophil and basophil typed into type I and type II categories.

oval, paced eccentrically as reported by [20] in cattle. Nucleus had fine lightly stained chromatin material (Fig.2) as finding of [16] in chicken and [8] in buffalo. The cytoplasm was diffusely granulated with fine granules; this is in agreement with finding of [18] in sheep. The average nuclear diameter in immature males was  $4.50 \pm 0.15 \mu\text{m}$ . The aforesaid value in immature female, mature male and mature female was  $4.50 \pm 0.15 \mu\text{m}$ ,  $5.64 \pm 0.14 \mu\text{m}$  and  $5.64 \pm 0.14 \mu\text{m}$  respectively (Table 2).

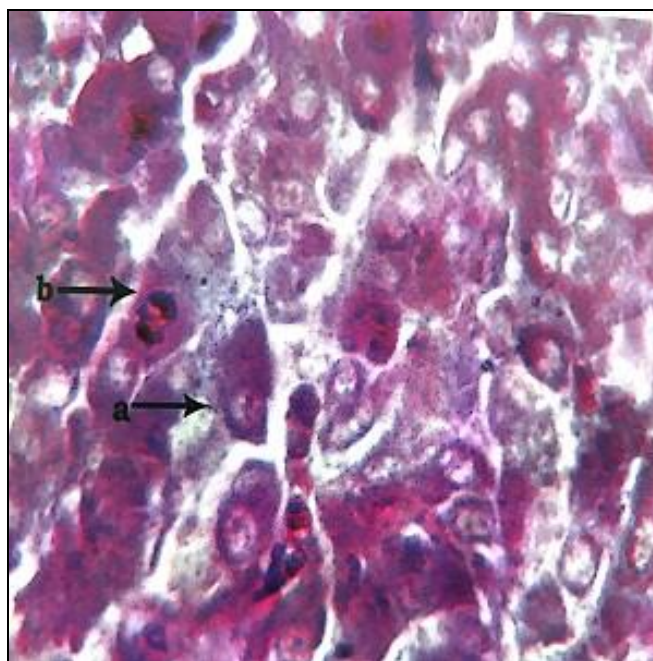
The average cell diameter in immature males was  $11.80 \pm 0.28 \mu\text{m}$ . This value in immature female was  $11.97 \pm 0.31 \mu\text{m}$ , in mature male was  $12.71 \pm 0.34 \mu\text{m}$  and in mature female was  $12.71 \pm 0.46 \mu\text{m}$  (Table 3). The average cell and nucleus diameter were observed that increased in mature

than immature animals but no variation found among sexes. According to <sup>[18]</sup> the cell diameter was 12.6-14.2µm in sheep. The cell diameter increased with age in present finding as accordance with findings of <sup>[8]</sup> Khan in buffalo. The average distribution of Type I acidophil cells were 65.51%, 65.42%, 56.63% and 44.78% in immature male, immature female, mature male and mature female respectively (Table 4). The distribution of Type I acidophil was more in immature rabbit than mature rabbits. Among the mature rabbits this value was less in female, this findings were agreed with <sup>[8]</sup>. In present investigation the Type I acidophils were largest among the chromophils, which tally with findings of <sup>[8]</sup> who stated that the average diameters among the acidophil were largest for the GH cells in buffalo.

### Type II Acidophils

Cells were rounded, irregular polygonal and rarely elongated with densely granulated cytoplasm. The nuclei were usually rounded or oval eccentrically placed. Sometime centrally placed nuclei were also seen. The nuclei contained coarse granular chromatin material which was irregularly distributed

(Fig.2). This findings regarding shape of cell and granular cytoplasm are in agreement with those of <sup>[14]</sup> in sheep and <sup>[16]</sup> in chicken. The average nuclear diameter in immature male and female was  $4.50 \pm 0.15 \mu\text{m}$  and the said value in mature female and male was  $5.64 \pm 0.14 \mu\text{m}$  (Table 2). The average nuclear diameter was observed to be increased in mature rabbits than immature ones and remain same in both sexes. The average cell diameter in immature male was  $10.66 \pm 0.32 \mu\text{m}$  and immature female was  $10.77 \pm 0.32 \mu\text{m}$ . In mature male and female said value was  $11.06 \pm 0.33 \mu\text{m}$  and  $11.17 \pm 0.34 \mu\text{m}$  respectively (Table 3). The average cell diameter was detected to be increased in mature rabbit than immature rabbit and in between sex it remained same. This finding is almost in accordance with <sup>[14]</sup> in sheep and <sup>[9]</sup> in buffalo. In present investigation detected the Type I acidophils was larger than Type II acidophil as findings of <sup>[14]</sup> in sheep. The average distribution of type II acidophils remained almost same in immature male, immature female and in mature male but this cell percentage increased in mature female (Table 4). This finding agreed with <sup>[9]</sup> in buffalo.



**Fig 2:** Photomicrograph of pituitary gland of mature male rabbit showing pars distalis, a- Type-I Acidophils, b- Type-II Acidophils. Azan X1000

### Basophil

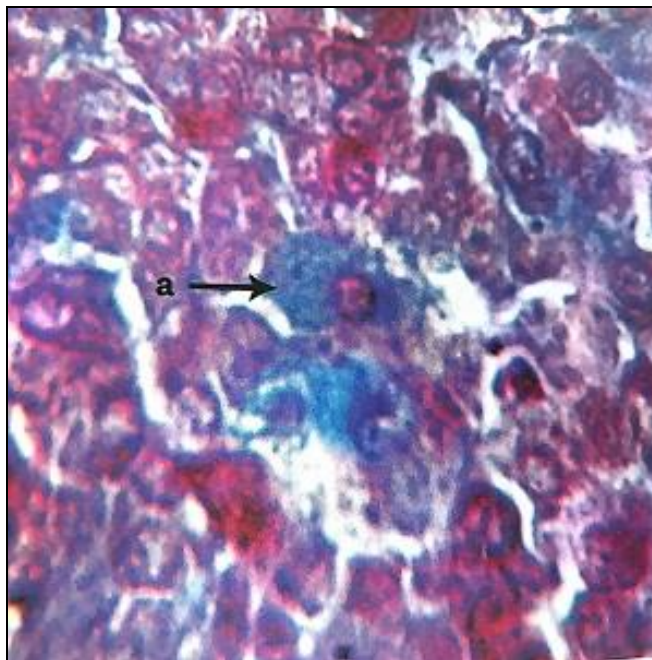
These cells were rounded or oval, polygonal, narrow and elongated. They occurred singly or in groups. The cytoplasm was discretely granular and nuclei were eccentrically. This observation is supported by <sup>[18]</sup> in sheep and <sup>[13]</sup> in Red sheep. The average cell distribution irrespective of type of cell was 6.36% in immature male, 7.32% in immature female, 14.75% in mature male and 14.94% in mature female (Table 1). According to findings of <sup>[10]</sup> the basophil was 15% of total cell population of the gland in human.

### Type I basophil

The cells were generally polygonal, elongated and rarely oval with eccentrically placed rounded or oval nucleus. The nuclei

contain very fine highly stained chromatin materials. The cytoplasm was densely granulated with coarse granules (Fig. 3). This finding agreed with <sup>[16]</sup> in chicken. The average diameter of nucleus and cells in immature male was  $4.67 \pm 0.14 \mu\text{m}$  and  $9.8 \pm 0.23 \mu\text{m}$  respectively. The said value in immature females was  $4.67 \pm 0.14 \mu\text{m}$  and  $9.8 \pm 0.23 \mu\text{m}$  respectively. In mature males the values were  $5.76 \pm 0.15 \mu\text{m}$  and  $11.57 \pm 0.28 \mu\text{m}$  and in mature female they were  $5.76 \pm 0.15 \mu\text{m}$  and  $11.17 \pm 0.28 \mu\text{m}$  respectively (Table 2 and 3). The average nuclear diameter was found to be increased in mature animals than immature ones and in between sexes they remained almost same as findings of <sup>[18]</sup> in sheep. The average distribution of type I basophil was observed to be decreased with age but remained same in both the sexes (Table 4).





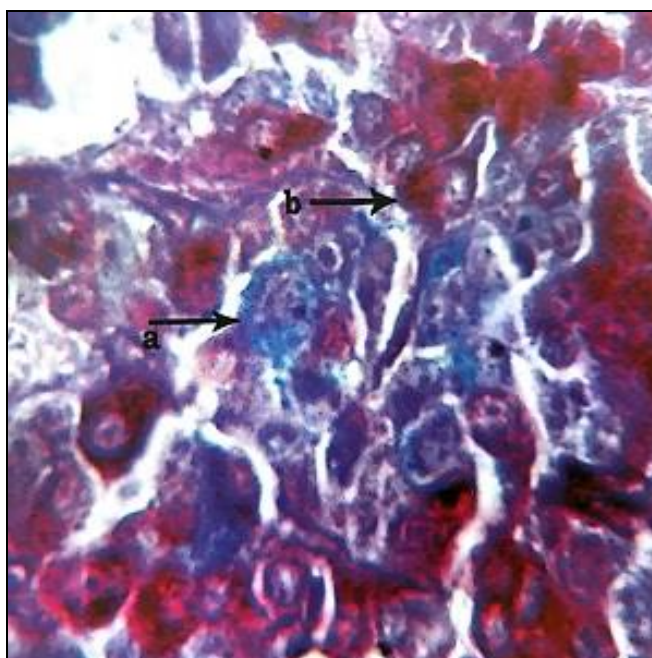
**Fig 3:** Photomicrograph of pituitary gland of mature female rabbit showing pars distalis, a- Type-I Basophil.

Azan X1000

#### **Type II basophil**

The cells are irregularly rounded, oval or angular. The nuclei were rounded or oval lightly stained and generally eccentrically placed. The cytoplasm was fine granular (Fig.4). This is in accordance with <sup>[16]</sup> in chicken and <sup>[12]</sup> in musk shrew. The nuclear diameter in both immature male and female was  $4.39 \pm 0.16 \mu\text{m}$  where as in mature male and mature female the value was  $5.36 \pm 0.12 \mu\text{m}$  (Table 2). The cell diameter in immature male and immature female was

$8.95 \pm 0.18 \mu\text{m}$  and  $8.91 \pm 0.18 \mu\text{m}$  respectively. The said values in mature male and female were  $9.35 \pm 0.22 \mu\text{m}$  and  $9.29 \pm 0.21 \mu\text{m}$  respectively (Table 3). The average distributions of Type II basophil in immature male, immature female mature male and mature female were 2.04%, 2.49%, 16.36% and 15.89% respectively of total chromophils in a unit area in the pars distalis (Table 4). This observation confirms the finding of <sup>[15]</sup>.



**Fig 4:** Photomicrograph of pituitary gland of mature female rabbit showing pars distalis, a- Type-II Basophil, b- Type-I Acidophils.

Azan X1000

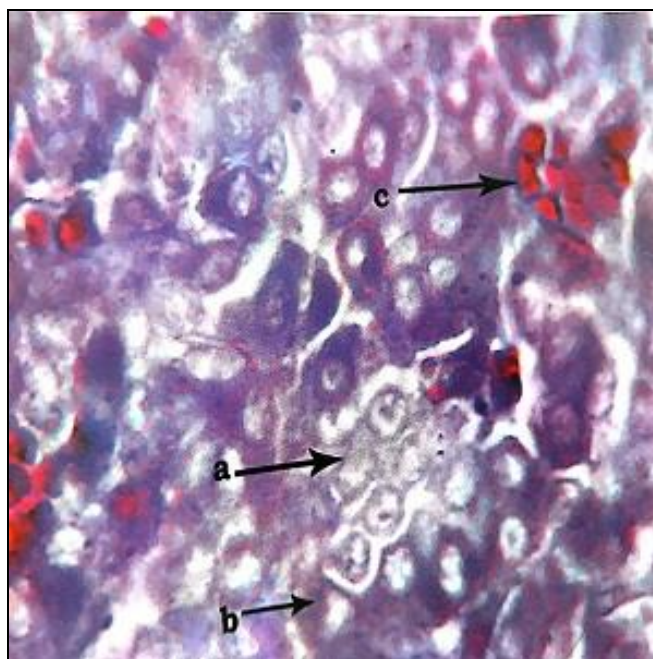
#### **Chromophobes**

The cytoplasm of these cells stained very weakly and was not well outlined. They were found sparsely distributed throughout the pars distalis. They were distributed in clumps or in groups of 5-6 cells or more. Their nuclei were rounded

and lightly stained (Fig.5). The size of chromophobes was smallest out of all the cells of adenohypophysis pars distalis. This is in agreement to the statement of <sup>[5]</sup> in buffalo. The average cell and nuclear diameter in immature male was  $7.59 \pm 0.21 \mu\text{m}$  and  $4.33 \pm 0.18 \mu\text{m}$  respectively. The said value

in immature female was  $7.07 \pm 0.21 \mu\text{m}$  and  $4.33 \pm 0.18 \mu\text{m}$ , in mature male was  $8.21 \pm 0.28 \mu\text{m}$  and  $5.36 \pm 0.12 \mu\text{m}$  and in mature female was  $8.78 \pm 0.22 \mu\text{m}$  and  $5.36 \pm 0.12 \mu\text{m}$  respectively (Table 2 and 3). The result on nuclear and cell diameter showed that it increased with age and remained same in both sexes. This is as per with the report of [15]. The average distribution of these cell in immature male and female was 49.53% and 48.92% respectively. In mature male and female these percentage were 39.98% and 36.80% respectively (Table 1). The average percentage of these cells

decreased with increase of age and remained almost equal in either sex as reported by [16] in chicken. The decrease in the percentage of chromophobes and increase in percentage of basophil with the advancement of age indicates that the chromophobes might be differentiated into basophil during this period. This confirms the statement of [4] who have recorded that in human, the chromophobes were reserve cells capable of differentiating into either acidophil or basophil.



**Fig 5:** Photomicrograph of pituitary gland of mature male rabbit showing pars distalis, a- cluster of chromophobes, b- cords of acidophils c- RBC.

Azan X1000

**Conclusion**

The cells of adenohypophysis pars distalis in rabbits were arranged either in the form of cords or in the form of acini. All the chromophils were classified into Type I and Type II categories on the basis of histomorphological character. The percentages of chromophobes were decreased and the percentage of basophils increased with advancement of age of rabbits. However, acidophils percentage was 45.27% in mature male and 48.26% in mature female, whereas in immature rabbit it was more or less similar in of both sexes.

**Table 1:** The relative proportion in percentage of acidophil, basophil and chromophobe in the pars distalis in different groups of rabbit

| Groups          | Percentage of cell types |           |              |
|-----------------|--------------------------|-----------|--------------|
|                 | Acidophils               | Basophils | Chromophobes |
| Immature male   | 44.11                    | 6.36      | 49.53        |
| Immature female | 43.76                    | 7.32      | 48.92        |
| Mature male     | 45.27                    | 14.75     | 39.98        |
| Mature female   | 48.26                    | 14.94     | 36.80        |

**Table 2:** The nuclear size (average diameter) of different types of cell in pars distalis in different groups of rabbit.

| Groups          | Average cell diameter of cell types in ( $\mu\text{m}$ ) |                    |                  |                   |                 |
|-----------------|--|--------------------|------------------|-------------------|-----------------|
|                 | Type – I Acidophil                                       | Type –II Acidophil | Type –I Basophil | Type –II Basophil | Chromophobes    |
| Immature male   | $4.50 \pm 0.15$  | $4.50 \pm 0.15$    | $4.67 \pm 0.14$  | $4.39 \pm 0.16$   | $4.33 \pm 0.18$ |
| Immature female | $4.50 \pm 0.15$  | $4.50 \pm 0.15$    | $4.67 \pm 0.14$  | $4.39 \pm 0.16$   | $4.33 \pm 0.18$ |
| Mature male     | $5.64 \pm 0.14$  | $5.64 \pm 0.14$    | $5.76 \pm 0.15$  | $5.36 \pm 0.12$   | $5.36 \pm 0.12$ |
| Mature female   | $5.64 \pm 0.14$  | $5.64 \pm 0.14$    | $5.36 \pm 0.12$  | $5.36 \pm 0.12$   | $5.36 \pm 0.12$ |

**Table 3:** The cell size (average diameter) of different types of cell in pars distalis in different groups of rabbit

| Groups          | Average cell diameter of cell types in ( $\mu\text{m}$ ) |                    |                  |                   |                 |
|-----------------|--|--------------------|------------------|-------------------|-----------------|
|                 | Type – I Acidophil                                       | Type –II Acidophil | Type –I Basophil | Type –II Basophil | Chromophobes    |
| Immature male   | $11.80 \pm 0.28$   | $10.66 \pm 0.32$   | $9.8 \pm 0.23$   | $8.95 \pm 0.18$   | $7.59 \pm 0.21$ |
| Immature female | $11.97 \pm 0.31$   | $10.77 \pm 0.32$   | $9.8 \pm 0.23$   | $8.91 \pm 0.18$   | $7.07 \pm 0.21$ |
| Mature male     | $12.71 \pm 0.34$   | $11.06 \pm 0.33$   | $11.57 \pm 0.28$ | $9.35 \pm 0.22$   | $8.21 \pm 0.28$ |
| Mature female   | $12.71 \pm 0.46$   | $11.17 \pm 0.34$   | $11.17 \pm 0.28$ | $9.29 \pm 0.21$   | $8.78 \pm 0.22$ |

**Table 4:** The distribution (as percentage of chromophils) of different cell types of acidophils and basophil of the pars distalis in different groups of rabbit.

| Groups          | Percentage of cells type |                    |                  |                   |
|-----------------|--------------------------|--------------------|------------------|-------------------|
|                 | Type – I Acidophil       | Type –II Acidophil | Type –I Basophil | Type –II Basophil |
| Immature male   | 65.51                    | 21.48              | 10.61            | 2.04              |
| Immature female | 65.42                    | 19.99              | 12.10            | 2.49              |
| Mature male     | 56.63                    | 18.81              | 8.20             | 16.36             |
| Mature female   | 44.78                    | 31.60              | 7.75             | 15.89             |

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