Dynamics of morphometric parameters of α-endocrinocytes of mammal pancreas

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Abstract. In domestic animals in postnatal ontogenesis, statistically significant changes in the number of α-endocrinocytes and the values of their nuclear-cytoplasmic ratio in the endocrine islets of pancreas have been revealed, which reflects the species and age-related dynamic processes of its morphofunctional development in animals. In cattle and small cattle (sheep), pigs, dogs and cats, two critical periods of postnatal development of the pancreas have been identified. The first critical period is observed from the birth to the age of three months. It is associated with an alimentary factor: the transition from a dairy diet to roughage, which leads to a decrease in the values of morphometric indicators of the pancreas and the restructuring of the functional work of the organ as a whole. The second critical period is registered in animals during puberty, that is, at 6 months of age, which is associated with the release of hormonal inducers aimed primarily at the implementation of physiological changes in the body.

1 Introduction

The pancreas has always been of interest to scientists around the world [1]. The pancreas is an important part of the digestive tract that performs exocrine and endocrine functions, controlling energy metabolism throughout the body. Endocrine portions comprise 1-2% of pancreatic mass. Different types of islet cells secrete the hormones insulin (β cells) glucagon (α cells), somatostatin (δ cells), and pancreatic polypeptide (PP cells) [2, 3].

Islet cells, especially β cells, are being actively studied in connection with the search for new approaches in the treatment of various diseases of the pancreas, such as cancer and diabetes ]4-10].

It is known that α-endocrinocytes produce glucagon, which increases glycogen catabolism, activates gluconeogenesis, lipolysis and ketogenesis in a liver, insulin secretion, cyclic adenosine monophosphate, catecholomin-medullary substance of the adrenal glands, and inhibition of insulinase. Under the action of glucagon, arterial pressure and relaxation of smooth musculature of internal organs increases [11, 12].

Glucagon-containing secretory material in the cell is detected in the form of rounded formations being a homogeneous substance of very high electron density. When identifying

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α-cells, the degree of secretion osmophilicity and a narrow light rim between the membrane and the heart-shaped granule are taken into account [13, 14].

The pancreas of all mammals has a similar structure, but a specialized diet is accompanied by a number of morphological features [15].

2 Materials and Methods

Domestic animals were selected as the subjects to study: from the mammal class – Ayrshire cattle, small cattle, sheep of Stavropol breed, German and Russian piebald hound dogs, Thai and British cat breeds, and a large white breed of pigs.

300 male animals of different age groups and species were selected to study. Each age group included males at different stages of postnatal ontogenesis: 1-day (n=10); 1-month (n=10); 3-months (n=10); 6-months (n=10); 1 year (n=10); 3 years (n=10).

The study was conducted in accordance with Directive 2010/63 / EU of the EUROPEAN PARLIAMENT and the COUNCIL of the EUROPEAN UNION on the protection of animals used for scientific purposes.

The data for study was the pancreas from which histological specimens were prepared using immunohistochemical method with a set of monoclonal muscular antibodies to glucagon (DiagnosticBioSystems, the Netherlands, 1:25 – 1:50). Histologic specimen microscopy was performed by using an Olympus BX45 digital microscope with a built-in C 300 camera (Japan). 10 digital images of random fields of view were taken from each pancreas specimen at a magnification of ×1000. Morphometric studies were performed using the programme of VideoTest-Master Morphology 4.0 for Windows (Russia).

The nuclear-cytoplasmic ratio (NCR) in the studied cells was calculated using the following formula: NCR = Sn/Sc, where: Sn – the area of the cell nucleus; Sc - the area of the cytoplasm. The obtained results were statistically processed using single-factor analysis of variance and Newman-Casle multiple comparison criterion in the «Primer of Biostatistics 4.03» programme for Windows. Significant differences were considered at p≤0.05.

Purpose of research. To determine the dynamics of changes in the number and nuclear-cytoplasmic ratio of α-endocrinocytes in mammal endocrine islets.

3 Results

Under a comparative analysis of the number of α-endocrinocytes in the endocrine islets of pancreas among animals at the age of one day, it was found that the peak value of this indicator is registered in pigs, and the minimum value is registered in sheep (Table 1).

In pigs at the age of a day, the number of α-endocrinocytes is greater than in sheep by 2.30 times, in dogs – by 18.37 %, in cats – by 42.27 %, respectively. In cattle, the value of this indicator is greater than in sheep – by 2.13 times, in sheep – by 2.32 times, in sheep – by 31.64 %, respectively. In sheep, the number of α-endocrinocytes is less than in dogs by 95.22 %, in cats – by 62.42% respectively. In dogs, the value of this indicator is greater than in cats by 20.20 %.

There were no significant differences in the number of α-endocrinocytes between the other studied species of animals at the age of a day.

At the age of a month, the highest value of the number of α-endocrinocytes is recorded in pigs, and the lowest value – in sheep. In pigs, the value of this indicator is higher than in cattle by 72.55 %, in sheep – by 2.32 times, in dogs – by 43.07 % and in cats – by 65.18 %, respectively. In cattle at the age a month, the number of α-endocrinocytes is higher than in sheep – by 35.0 %. In sheep, the value of this indicator is less than in dogs by 62.82 % and
in cats – by 41.03 %, respectively. The values of the number of α-endocrinocytes do not differ significantly between the other studied species of animals at the age of a month.

**Table 1.** The number of α-endocrinocytes in the endocrine islets of the pancreas of animals in postnatal ontogenesis, units.

| Age     | Animal species | Cattle (M±m) | Speep (M±m) | Pigs (M±m) | Dogs (M±m) | Cats (M±m) |
|---------|----------------|--------------|-------------|------------|------------|------------|
| 1 day (n=100) |                | 16.10±1.14  | 7.53±0.46  | 17.40±0.71 | 14.70±0.65 | 12.23±0.66 |
| 1 month (n=100) |               | 10.53±0.79* | 7.80±0.69  | 18.17±1.48 | 12.70±0.61 | 11.0±0.52* |
| 3 months (n=100) |              | 14.60±1.21* | 11.37±0.62* | 15.50±0.67 | 11.20±0.62 | 24.20±2.79* |
| 6 months (n=100) |                | 11.97±1.07* | 19.57±0.58* | 11.23±0.81* | 5.36±0.69* | 13.40±1.41* |
| 1 year (n=100) |                   | 9.06±0.61   | 8.53±0.68* | 11.40±0.76 | 8.57±0.42* | 15.27±2.15* |
| 3 years (n=100) |                  | 8.80±0.51   | 9.90±0.63  | 22.07±1.17* | 14.33±0.56* | 11.03±0.54* |

Note: the statistical significance of differences (at p≤0.05) in earlier age is indicated by *.

In three-month-old animals, the number of α-endocrinocytes in the endocrine islets has the maximum values in cats, and the minimum values – in sheep and dogs. In cats, the value of this indicator is greater than in cattle by 65.75 %, in sheep – by 2.12 times, in pigs – by 56.13 % and in dogs – by 2.16 times, respectively. There were no significant differences in the number of α-endocrinocytes between the other studied animal species.

The comparative analysis of the number of α-endocrinocytes in the studied animal species at the age of six months showed that the highest value of this indicator was registered in sheep, and the lowest one – in dogs. In six-month-old sheep, the number of α-endocrinocytes is higher than in cattle by 63.49 %, in pigs – by 74.27 %, in dogs – by 3.64 times, in cats – by 46.04 %, respectively. In cattle, the value of this indicator is greater than in pigs by 6.59 % and in dogs – by 2.23 times, respectively. In dogs, the number of α-endocrinocytes is less than in pigs by 2.09 times, in cats – by 2.49 times, respectively. There were no significant differences in the number of α-endocrinocytes in cattle, pigs, and cats at the age of 6 months.

At the age of a year, the number of α-endocrinocytes in animals has the peak value in cats, and the minimum value in sheep. In cats, the value of this indicator is greater than in cattle by 68.58 %, in sheep – by 79.02 %, in pigs – by 33.95 %, in dogs – by 78.18 %, respectively. There were no significant differences in the number of α-endocrinocytes between the other studied animal species at the age of one year.

At the age of three years of the studied animal species, the number of α-endocrinocytes has the peak value in pigs, and the minimum value – in cattle. In pigs, the value of this indicator is higher than in cattle by 2.50 times, in sheep – 2.22 times, in dogs – by 54.01 %, in cats – by 100.09 %, respectively. In dogs, the number of α-endocrinocytes is higher than in cattle by 62.84 %, in sheep – by 44.75 % and in cats – by 29.92 %, respectively.

Therefore, in cattle, the number of α-endocrinocytes has only minimal values at the age of three years compared with other animal species.

In sheep, the peak value of the number of α-endocrinocytes was registered from one-month to six-month age, but they also have minimum values of this indicator at the age of a day and a year compared to other animal species.

The highest values of the number of α-endocrinocytes are observed only in pigs. This indicator is recorded at the age of one day, one month and three years compared to other animal species.
Dogs at the age from 3 months to 6 months have only minimal values of the number of α-endocrinocytes compared to other animal species.

In cats, the peak indicators of the number of α-endocrinocytes were registered at the age of three 3 months and one year compared to other animal species.

There were no significant differences in the number of α-endocrinocytes between the other animal species studied.

Therefore, in cattle, the number of α-endocrinocytes has only minimal values at the age of three years, compared with other animal species. In sheep, the peak value of the number of α-endocrinocytes was registered from the age of one month to six months, but they also have minimum values of this indicator at the age of one day and one year compared to other animal species. In pigs, the highest values of the number of α-endocrinocytes are observed in 1-day, 1-month age and 3 years. In dogs, the highest values of the number of α-endocrinocytes are observed in 1-day and age and 3 years. In cats, the peak indicators of the number of α-endocrinocytes were registered at the age of three months and one year.

The comparative analysis of NCR of α-endocrinocytes among the animals at the age of one day and six months determined that the peak value of this indicator was registered in sheep and cattle, and the minimal one – in cats (Table 2).

In cattle at the age of one day, NCR of α-endocrinocytes is more than in pigs by 34.58 %, in dogs – by 29.73 %, in cats – by 38.46 %, respectively. In sheep, the value of this indicator is higher in comparison with pigs by 37.11 %, with dogs – by 27.93 % and with cats – by 36.54 %, respectively.

There were no significant differences in the number of α-endocrinocytes among the other animal species studied.

At the age of one month, NCR of α-endocrinocytes possesses the peak value in pigs, the least one – in dogs.

| Age  | Cattle (M±m) | Sheep (M±m) | Pigs (M±m) | Dogs (M±m) | Cats (M±m) |
|------|--------------|-------------|------------|------------|------------|
| 1 day (n=100) | 0.28±0.01 | 0.28±0.013 | 0.21±0.007 | 0.22±0.008 | 0.21±0.005 |
| 1 month (n=100) | 0.25±0.01 | 0.22±0.012* | 0.26±0.01* | 0.18±0.006* | 0.19±0.005 |
| 3 months (n=100) | 0.27±0.01 | 0.29±0.012* | 0.26±0.01 | 0.18±0.005 | 0.17±0.008 |
| 6 months (n=100) | 0.22±0.009* | 0.32±0.014 | 0.23±0.009* | 0.19±0.007 | 0.18±0.008 |
| 1 year (n=100) | 0.24±0.008 | 0.16±0.006* | 0.21±0.008 | 0.17±0.004 | 0.12±0.007* |
| 3 years (n=100) | 0.25±0.01 | 0.18±0.007 | 0.20±0.005 | 0.16±0.004 | 0.22±0.012* |

Note: the statistical significance of differences (at p≤0.05) with earlier age is indicated by *.

In pigs, the value of this indicator is higher compared to sheep – by 16.30 %, compared to dogs – by 44.26 % and cats – by 33.33 %, respectively. In cattle at the age of one month, NCR of α-endocrinocytes is higher than in sheep – by 31.22 %, in dogs – by 40.44 % and in cats – by 29.80 %, respectively. In sheep, the value of this indicator is higher compared to dogs – by 24.04 % and cats – by 14.65 %, respectively.

Among dogs and cats at the age of one month, the value of NCR of α-endocrinocytes of the endocrine islets of pancreas does not differ significantly.

In three-month-old males, NCR of α-endocrinocytes has the peak value in sheep, and the minimum value in cats.
In sheep, the value of this indicator is higher than in pigs – by 15.50 %, in dogs – by 61.96 % and in cats – by 72.25 %, respectively. In cattle, there are more NCR of α-endocrinocytes in dogs – by 48.37 %, and in cats – by 57.80 %, respectively. In pigs, the value of this indicator is higher than in dogs – by 40.22 %, and more than in cats – by 49.13 %, respectively. There were no significant differences in the values of NCR of α-endocrinocytes between dogs and cats.

The comparative analysis of NCR of α-endocrinocytes at the age of 6 months determined that the highest value of this indicator was registered in sheep, and the lowest one – in cats.

In six month-old sheep, NCR of α-endocrinocytes is higher than in cattle by 43.24 %, in pigs – by 40.10 %, in dogs – by 60.61 %, in cats – by 76.67 %, respectively. In cats, the value of this indicator is lower compared to cattle – by 23.33 % and pigs – by 26.11 %. There were no significant differences in the values of NCR of α-endocrinocytes between the other animals at the age of six months.

At the age of one year, NCR of α-endocrinocytes in cattle has the peak value, and the minimum value – in cats.

In cattle, the value of this indicator is higher than in sheep by 53.17 %, in pigs – by 16.90 %, in dogs – by 35.20 %, in cats – by 65.75 %, respectively. In pigs, NCR of α-endocrinocytes is higher than in sheep by 31.01 %, in dogs – by 15.64 %, in cats – by 41.78 %, respectively. Dogs at the age of one year have more NCR of α-endocrinocytes than sheep by 13.29 %, and cats – by 22.60 %, respectively. The value of this indicator does not differ significantly between sheep and cats.

At the age of 3 years, NCR of α-endocrinocytes has the peak value in cattle, and the minimum value – in dogs.

In cattle, the value of this indicator is higher than in sheep by 37.99 %, in pigs – by 21.68 %, in dogs – by 48.80 %, in cats – by 11.26 %, respectively. Cats at the age of three years have more NCR of α-endocrinocytes compared to rams – by 24.02 % and male dogs – by 33.74 %, respectively. In pigs, the value of this indicator is greater than in dogs by 22.29 %. There were no significant differences in the values of NCR of α-endocrinocytes between the other animals.

4 Conclusion

Therefore, in domestic animals in postnatal ontogenesis, statistically significant changes in the number of α-endocrinocytes and the values of their nuclear-cytoplasmic ratio in the endocrine islets of pancreas have been revealed, which reflects the species and age-related dynamic processes of its morphofunctional development in animals. In cattle and small cattle (sheep), pigs, dogs and cats, two critical periods of postnatal development of the pancreas have been identified. The first critical period is observed from the birth to the age of three months. It is associated with an alimentary factor: the transition from a dairy diet to roughage, which leads to a decrease in the values of morphometric indicators of the pancreas and the restructuring of the functional work of the organ as a whole. The second critical period is registered in animals during puberty, that is, at 6 months of age, which is associated with the release of hormonal inducers aimed primarily at the implementation of physiological changes in the body.

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