Characterization of the histological changes in ovaries of Goto-Kakizaki diabetic rats

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SUMMARY

Goto-Kakizaki (GK) rats are a useful animal model for studying type 2 diabetes mellitus (T2DM). However, this strain of rats exhibits poor fertility, so it is difficult to expand colonies. Based on previous studies, it was hypothesized that alterations in the oestrous cycle of diabetic rats are related to ovarian histology. The aim of this study was to determine the histology of the ovaries of diabetic rats compared to nondiabetic rats to understand the poor fertility of this strain of diabetic rats. An experimental study was thus conducted. Eight GK rats and eight Wistar rats were utilized. The rats were age-adjusted into two groups called "young rats" and "mature rats" at two and fourteen months of age, respectively. After sacrificing the rats, the ovaries were dissected and processed by fixation and paraffin embedding to perform the histological study. The results included the number of corpora lutea and the percentage of follicular fraction in each ovary, as well as qualitative data such as the presence of follicles in different stages of development. Our findings revealed differences between GK and Wistar rats. The ovarian histological findings were related to the presence of T2DM, polycystic ovary syndrome and poor fertility in female GK rats; the link between these pathologies is insulin resistance. Future lines of investigation into metabolic treatment, which may help improve insulin resistance, could also benefit the previously described pathologies in female GK rats.

Key words: Goto-Kakizaki rats – Type 2 diabetes mellitus – Ovary – Infertility – Ovulatory dysfunction – Polycystic ovary syndrome

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is much more than a single pathology; it is a chronic disease based on a pathophysiological process consisting of tissue resistance to insulin action (WHO, 2022). T2DM continues to increase in prevalence and incidence and is a leading cause of death (Khan et al., 2020). T2DM is not a unique pathology, as there are many papers describing metabolic disorders related to insulin metabolism. These disorders involve diseases such as atherosclerosis (Ng

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et al., 2021), neuropathy (Selvarajah et al., 2019), hypothalamic-pituitary endocrine axis dysregulation (Lechuga-Sancho et al., 2006), nephropathy (Prattichizzo et al., 2021) and ocular lesions (Teo et al., 2021). Our current work focuses on the final consequences of this hypothalamic dysregulation.

For these reasons, the high interest in the research fields related to T2DM is clearly observed in the literature. Researchers need good animal models for these purposes. Thus, an excellent animal model of insulin resistance with spontaneous nonobese T2DM was developed, known as the Goto-Kakizaki rat (GK). The GK rat strain exhibits features of T2DM that are very similar to those observed in humans (Nandy et al., 2010; Akash and Chen, 2013). Among other diabetic symptoms, GK rats exhibit reproductive dysfunction. This dysfunction poses a serious problem for preclinical research in animal production facilities, which have a reduced capacity to ensure the maintenance of research activity. Colonies used to be scarce and to have low genetic turnover.

In our experience and in the literature, these rats have a shortened fertile period and reduced litter sizes (Diaz and Balibrea, 2007). One study described menstrual changes in the oestrous cycle, with a longer proestrous period and a shorter oestrous period, in six-month-old GK rats (Pinto-Souza et al., 2016). In addition, the scientific literature has recently reviewed many distinctive metabolic disorders, such as polycystic ovary syndrome (Bourgneuf et al., 2021).

Hormonal disorders that cause alterations in the reproductive cycles of GK rats are part of the hormonal physiology that affects the ovary. The main objective of this study was to perform a quantitative and qualitative analysis of ovarian histology in diabetic and nondiabetic rats. The qualitative analysis includes a description of the ovarian parenchymal architecture and the presence of germinal structures, fibrosis and adipose tissue. The quantitative analysis reveals the percentage of the follicular area between diabetic and nondiabetic rats. Our purpose is to understand the pathophysiological basis of infertility in Goto-Kakizaki (GK) rats.

METHODS

Animals and ethics

Eight female Wistar rats and eight female GK rats weighing approximately 100-120 g at an age of 5-7 weeks were provided and kept at the Experimentation and Animal Production Service of University of Cadiz (SEPA). The animal procedures were performed with the approval of the University of Cadiz Committee for the Ethical Use and Care of Experimental Animals, and every experiment was performed in accordance with relevant international guidelines and regulations for animal welfare.

Eight GK rats were randomly divided into two groups of 4 animals each. The first group was sacrificed after two months of survival. The second group was sacrificed after fourteen months of survival. The same procedure was performed with the group of 8 Wistar rats. All animals were maintained in an environmentally controlled room under standard conditions: temperature of 20-21°C, humidity of 45-65% and a 12-h:12-h light/dark cycle, and the animals were fed standard laboratory rat chow and water ad libitum.

Experimental procedures

The animals were sacrificed by isoflurane inhalation overdose. The ovaries were then dissected and fixed in Bouin's solution overnight at 4°C. Later, the samples were dehydrated, embedded in paraffin and cut into 10-µm sections with a microtome.

Histological study

Twelve serial rehydrated sections from ovary samples of each timepoint were used to study the number of corpora lutea, and the percentage of follicular fraction was assessed using haematoxylin & eosin staining. A minimum of ten fields at each point of study were analysed by a single investigator using a microscope (Olympus, GmbH. Hamburg, Germany) with a digital camera and ImageJ software. Data obtained were expressed as the number of corpora lutea/mm^2 of ovarian parenchyma or the percentage of follicular fraction.

Statistical analysis

Data are presented as the means ±SEMs. For histological analysis, the Mann–Whitney U test was conducted using SPSS v21.0 software. Statistical significance was accepted at $P<0.05$ (*).

RESULTS

Qualitative analysis

In the ovaries of young rats, no differences in ovarian sections were observed between the two groups of rats. The ovaries showed good architectural organization with many follicles in different stages of development, primordial/unilaminar follicles, multilaminar follicles and secondary follicles, as well as the corpus luteum (Fig. 1). A clearly visible increase in the corpus luteum was observed in the sixteen-month-old rats. These corpora lutea were immersed in a disorganized parenchyma. Similar follicular structures were observed in both groups of rats (Fig. 2).

Quantitative analysis

The percentage of follicular area decreased in Wistar rats as a function of age (6.3% in the young group and 3.6% in the old group). However, in aged GK rats, an increase in the follicular fraction was observed when comparing the follicular area with respect to the total ovarian area (4.7% in the young group and 5.6% in the old group). Thus, in Wistar rats, the follicular area progressively decreased, whereas in GK rats, the follicular area did not seem to decrease. In contrast, the follicular area of GK rats appeared to be increased relative to extrafollicular histology. Otherwise, the follicular area was slightly reduced in young GK rats versus Wistar rats. However, the number of corpora lutea continued the natural course of ovarian physiology progression in Wistar rats (3 in the young group and 7 in the old group) and GK rats (3 in the young group and 5 in the old group), but the increase in GK rats was less than that in Wistar rats.

DISCUSSION

The differences in oestrous disorders shown between GK rats and Wistar rats (Pinto-Souza et al., 2016) should be correlated with ovarian microanatomy. To demonstrate the histological changes in GK rats compared with a control group, ovarian morphology was studied in the Wistar rat group, with expected results and normal ovarian physiology: the follicular area was smaller and the number of corpora lutea was higher in aged rats. However, the results in GK rats are different; in this group, the follicular area was larger in aged rats, possibly related to a recent article proposing a new approach to fertility problems in correlation with the development of polycystic ovary syndrome (PCOS) in GK rats (Bourgneuf et al., 2021).

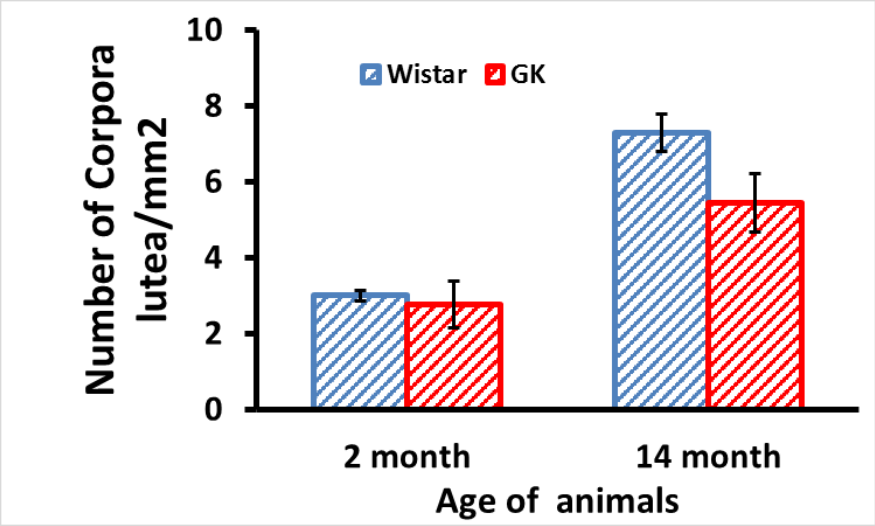

Fig. 1.- Analysis of the number of corpora lutea in Wistar (striped blue bar) and GK rats (striped red bar) at two and fourteen months after the start of the experiment. Age is represented on the X-axis, and the number of corpora lutea/mm^2 of ovarian parenchyma is represented on the Y-axis. Statistical significance at $p<0.05$ (*).

The aetiology of PCOS is still unclear. In the literature, two main physiological processes in this pathology are described: hormonal disorder and insulin resistance as a metabolic disorder (Hall, 2022).

GK rats are a good model animal to study T2DM because the insulin resistance present in these rats makes them diabetic rats. Furthermore, it should be emphasized that at this time we do not know whether hormonal disorders in female GK rats can cause molecular disorders in their future male offspring (Amaral et al., 2006).

Classically, in female subjects with PCOS, this disease has been established as the cause of metabolic complications. However, a new line of research could be formed to assess the hypothesis of the reversal of this argument based on the results of this study. Furthermore, due to the finding that 100% of GK rats developed PCOS, it may be interesting to know the specific genes that are modified in this species (Liu et al., 2015), as this could have a direct relationship with the development of PCOS and could ultimately be the origin of T2DM or vice versa. It would be interesting to contrast studies or develop new research in which the evolution of ovarian morphology and histology could be observed after prolonged periods of metabolic control.

Nevertheless, we need to incorporate a new perspective. In fertility reports about this strain, it is typical to find different methods to increase the colony when studying male rat conditions. No studies have been found about the fertility conditions or capabilities of male members of the GK rat strain. We proposed that this perspective has to be broadened. Male rats suffer from the same metabolic consequences of diabetic disorders. Thus, seminal, hormonal or sperm deficits should be considered when trying to improve fertility in these colonies. No studies have analysed fertility in male GK rats. As on many occasions, female rats initially represent the source of the infertility.

Two types of therapeutic alternatives could be assessed in female GK rats. To the best of our knowledge, there are no reports about the use of conventional insulin treatment to control glycaemic parameters. Otherwise, a hormonal-substitutive treatment related to PCOS could also be probed in colonies of this strain. The fertility rate is a parameter that provides information about the efficacy of these treatments.

Colonies of GK rats used to be complicated to maintain, which causes severe problems in diabetes research studies. The GK strain is a well-defined tool to study T2DM. As a genetically conditioned homeostasis, GK rats have been employed to study glucose- dependent peripheral resistance disorders in many research reports. Other gene-related strains, such as BB rats, are immu-

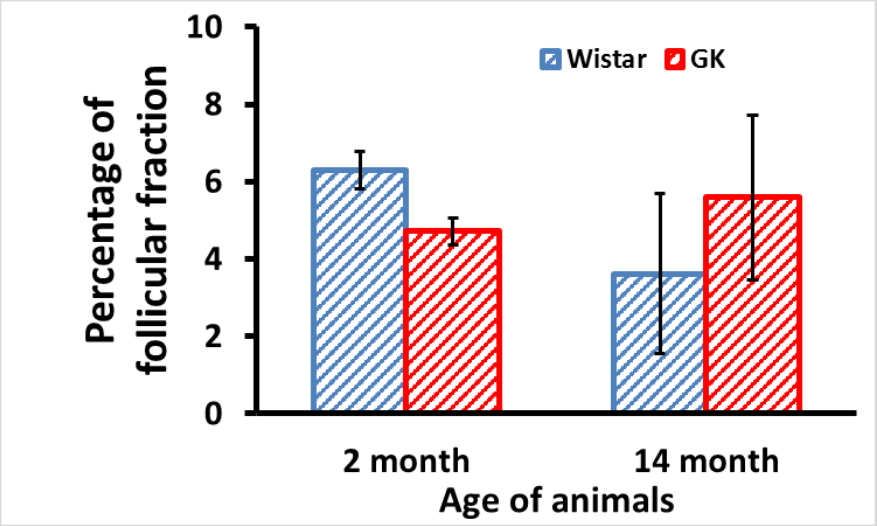

Fig. 2.- Percentage of the follicular fraction in Wistar (striped blue bar) and GK rats (striped red bar) at two and fourteen months after the start of the experiment. Age is represented on the X-axis, and the percentage of follicular fraction is represented on the Y-axis. Statistical significance at $p<0.05$ (*).

nologically conditioned to develop type 1 diabetes mellitus.

In our experience, according to several research groups in Spain using this strain of rat, the main problem in any research design is the modelling. Specifically, the sample size must be accounted for and the animals to be used must be prepared. Animal production services have difficulties maintaining requests for GK rats to meet the demands of research groups. Thus, any effort to improve the fertility of these colonies in animal production services would be welcome.

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