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Effect of Probiotic on the Pax2 Gene Expression during Kidney Development in Mice Embryos

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Annotation: Background & aim: At several stages of kidney and urinary tract development, Pax2 and Pax8 have become important participants in the urogenital system. Therefore, the aim of this work was to evaluate the effect of probiotic on the pax2 gene expression during kidney development in mice embryos.

Materials & methods: 30 experimental animals (Swiss mice) were utilized, three month-aged and weight ranged between 16-19gm at period April 2025. An acidophilus plus capsule (Solgar, USA) contains lactobacilli culture that injected (intraperitoneal) as two doses: $9*10^6$/0.1 ml CFU and $18*10^6$/0.2 ml CFU during pregnancy and then the fetal kidneys of these two group compare with control in terms of histology and molecular aspect.

Results: Microscopic examination of fetal kidneys in the probiotic $9*10^6$/0.1 ml group showed normal structures. However, $9*10^6$/0.1 ml group demonstrated greater development in the structure of the nephron compared to the control group. The number of glomeruli was greater and more rapidly developed, and the differentiation of the convoluted tubules was faster than in the control group. Furthermore, the capsule surrounding the kidney was better developed. Similarly, in the probiotic $18*10^6$/0.1 ml group, the development of the nephrons was more pronounced than in the control group, and the differentiation of the convoluted tubules and the capsule surrounding the kidney was more

distinct and clear. on the other hand, the finding showed that gene expression in studied groups, the mean of folding changes in control group was 0.9325. in 9*106/0.1 ml probiotic group folding changes was 0.9667. in 18*106/0.1 ml probiotic group folding changes was 1.021. The results showed non-significant (P=0.001) changes in gene expression between studied groups.

Conclusions: its concluded that the use of probiotics improves kidney development in fetuses, as the nephrons and urinary convoluted tubules were more developed and differentiated compared to the control group and improved expression of the *Pax2* gene in fetal kidneys.

Keywords: Kidney, probiotic, Pax2, gene expression.

Introduction

The gene PAX2 is a member of a gene family that is essential for the development of tissues and organs during embryonic life [1]. The preservation of some cells' proper function after birth also depends on the PAX gene family members. The PAX genes accomplish these functions by directing the synthesis of proteins that bind to particular regions of DNA and aid in regulating the activity (expression) of specific genes. PAX proteins are referred regarded as transcription factors because of their activity [2,3]. A protein important in the development of central nervous system, kidneys, the urinary system, and eyes is produced according to instructions provided by the PAX2 gene during embryonic development. It is believed that at times of cellular stress after birth, the PAX2 protein prevents cell death [4,5]. Probiotics are generally understood to be a group of live microorganisms that, when given in sufficient quantities, boost the host's health. Other bacterium-secreted substances or inactivated microorganisms that might have immunomodulatory qualities are not included in this description [6,7]. Probiotics work in the gastrointestinal tract by up-regulating and down-regulating genes linked to inflammation [8]. Most probiotics on the market belong to the Lactobacillus or Bifidobacterium genera and can modulate the immune system directly or indirectly. In the small intestine, where the population of commensal bacteria is smaller, the direct method, for example, utilizes cell surface receptors (TLR, CLR) [9,10]. Several major processes underpinning the positive benefits of probiotics include the alteration of the gut microbiota, the competitive adhesion to the mucosa, the improvement of the intestinal epithelial barrier and the modulation of the immune system and inflammation. The majority of these processes entail controlling gene expression in certain organs, including the liver and intestine [11,12]. In this regard, the regulation of gene expression by probiotics is a significant problem that requires attention. Probiotics may have an impact on mucin gene (MUC) expression. Commensal bacteria can also influence genes related to apoptosis-related enzymes, pro-inflammatory transcription factors, cytokines, and toll-like receptor (TLR) and nucleotide-binding oligomerization domain (NOD) receptors [12,13]. Therefore, the aim of this work was to evaluate the effect of probiotic on the pax2 gene expression during kidney development in mice embryos.

Materials & methods

Strains of bacteria

An acidophilus plus capsule (Solgar, USA) contains lactobacilli culture, which usually contains two billion L. acidophilus, L. casei casei, and L. casei rhamnosus. The culture of these lactobacilli strains was diluted in normal saline to include $9*10^6$ /0.1 ml CFU of acidophilus plus probiotics after being cultivated for 24 hours in nutrient broth medium. The second dilution in normal saline contained $30*10^3$ /0.1 ml CFU of acidophilus plus probiotics [14,15].

Laboratory animals

30 experimental animals (Swiss mice) were utilized, three month-aged and weight ranged between 16-19gm at period April 2025.

Experimental design

After confirming pregnancy, the probiotic was injected every 72 days, 6 times during the pregnancy period. The mice were dissected and the embryos were extracted on the 18th day of pregnancy. 30 mice were used in this study and then divided as follow (8 females and 2 males in each group)

- ✓ Control group received standard pellet diet only.
- ✓ Female mice injected (intraperitoneal) with probiotic acidophilus plus $9*10^6$/0.1 ml CFU.
- ✓ Female mice injected (intraperitoneal) with probiotic acidophilus plus $18*10^6$/0.2 ml CFU.

Histology samples

The animals were given chloroform anesthesia at the conclusion of the period, and the fetal kidneys were removed and fixed in 10% formalin for the night. The kidneys underwent ethanol dehydration, xylene removal, and paraffin wax embedding. A microtome was used to cut sections of the paraffin-embedded kidneys. Hematoxylin and eosin was used to stain the sections. Each section was covered with a cover slip, a drop of Canada balsam was applied, and the area was left to dry. The sections were ready to be examined under a light microscope. At a specific magnification, photographs were taken [16,17].

RNA extraction

The TRIzol Reagent was used to homogenize the fetal liver in order to extract the RNA. the present study extracted RNA using an RNA extraction kit called Transzol UP, following the instructions provided by the manufacturer in the protocol guidebook. The mRNA that was taken out was converted into cDNA through a process involving reverse transcriptase enzyme and a kit based on the instructions provided by the manufacturer. To prepare the primer for use, the experiment was left at 25°C for 10 minutes. Then, combine it with the EasyScript RT/RI Enzyme Mix before being incubated at 42°C for 15 minutes to facilitate transcription of the RNA sample. The enzyme is then deactivated by heating the reaction mixture to 85°C for 5 minutes in preparation for RT PCR analysis. Primers were utilized in conjunction with housekeeping genes for calibration to measure the levels of gene expression accurately. Primers were designed using forward and reverse sequences, as shown in Table 1.

Table 1. Primers designed for qPCR

Primer	Sequences	Ref.
Pax2 F	ATTCCTCGCTCCAACGGTGAGA	[18]
Pax2 R	CAGACCAGATGTAAACCTCCACC	
GAPDH-F	GAGATGTGTCTGAGGGCTCTG	
GAPDH-R	GTAACTAGTGGCGGTCATAGGC	

The following PCR program was used: Stage 1: Activation: 50 °C for 2 min; Stage 2: pre-soak:95 °C for 10 min; Stage 3: Denaturation: 95 °C for 15 secs, annealing: 60°C for 1 min; Stage 4: Melting curve: 95°C for 15 secs, 60°C for 15 secs, 95°C for 15 secs.

Statistical analysis

The statistical significance differences seen between the groups (n=5; P≤ 0.05) can be assessed by an ANOVA. Using Gene Expression Folding (2^-ΔΔCt) values, the variations in Pax2 gene expression levels among the experimental groups were assessed.

Results & Discussion

The study was conducted to determine whether there was a link between probiotics and fetal mouse kidney tissue, and whether there was an effect on the gene expression of pax2 after extracting RNA from kidney tissue using RT-PCR. The results were as follows:

Histological study

Control group

The control group showed the normal structure and development of fetal kidney structures. Nephrons were found to be normal, consisting of glomeruli surrounded by Bowman's capsule, convoluted tubules, and regular parenchymal cells. Furthermore, the perinephric capsule, composed of connective tissue, was present (Figure 1).

Probiotic groups

Microscopic examination of fetal kidneys in the probiotic $9*10^6$/0.1 ml group showed normal structures. However, this group demonstrated greater development in the structure of the nephron compared to the control group. The number of glomeruli was greater and more rapidly developed, and the differentiation of the convoluted tubules was faster than in the control group. Furthermore, the capsule surrounding the kidney was better developed (Figure 2). Similarly, in the probiotic $18*10^6$/0.1 ml group, the development of the nephrons was more pronounced than in the control group, and the differentiation of the convoluted tubules and the capsule surrounding the kidney was more distinct and clear (Figure 3).

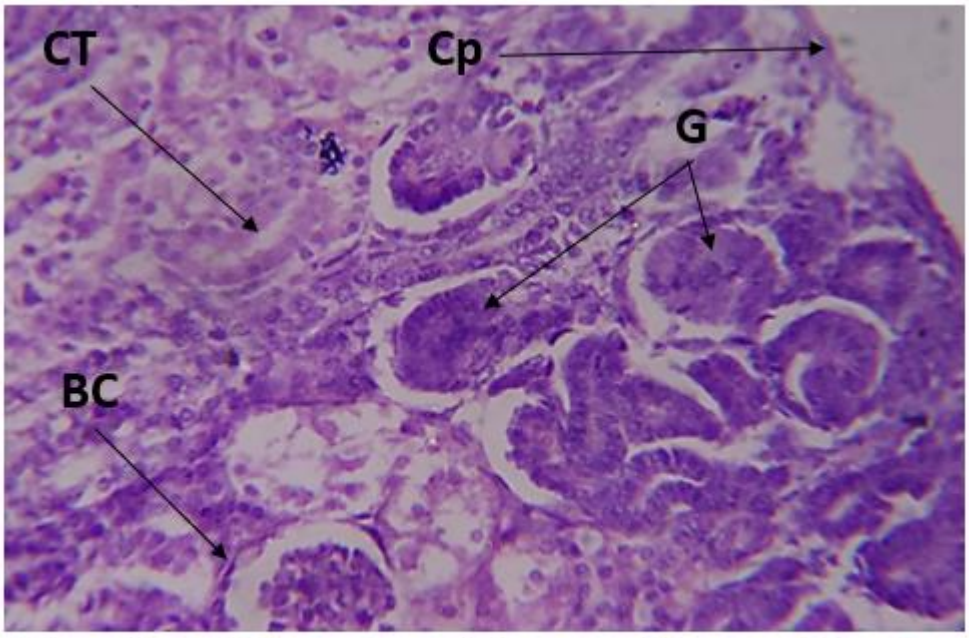

Figure (1): Fetal kidney of control demonstrated the normal formation of glomerulus (G), Bowman's capsule (BC) and convoluted tubules (CT) with capsule (Cp) H&E X400.

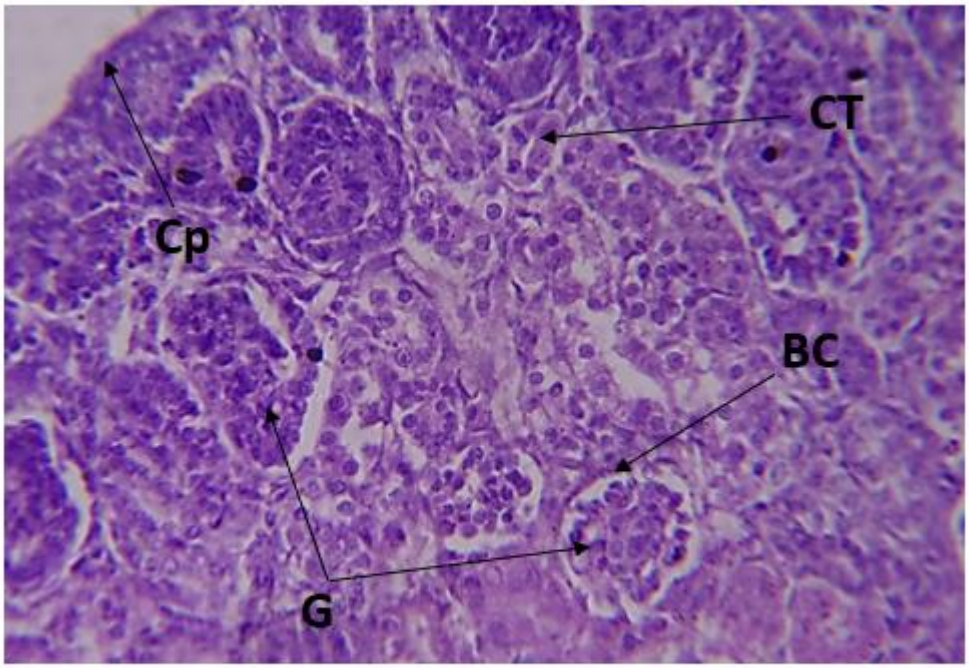

Figure (2): Fetal kidney of probiotic $9*10^6$/0.1 ml group demonstrated the normal formation of glomerulus (G), Bowman's capsule (BC) and convoluted tubules (CT) with capsule (Cp) H&E X400.

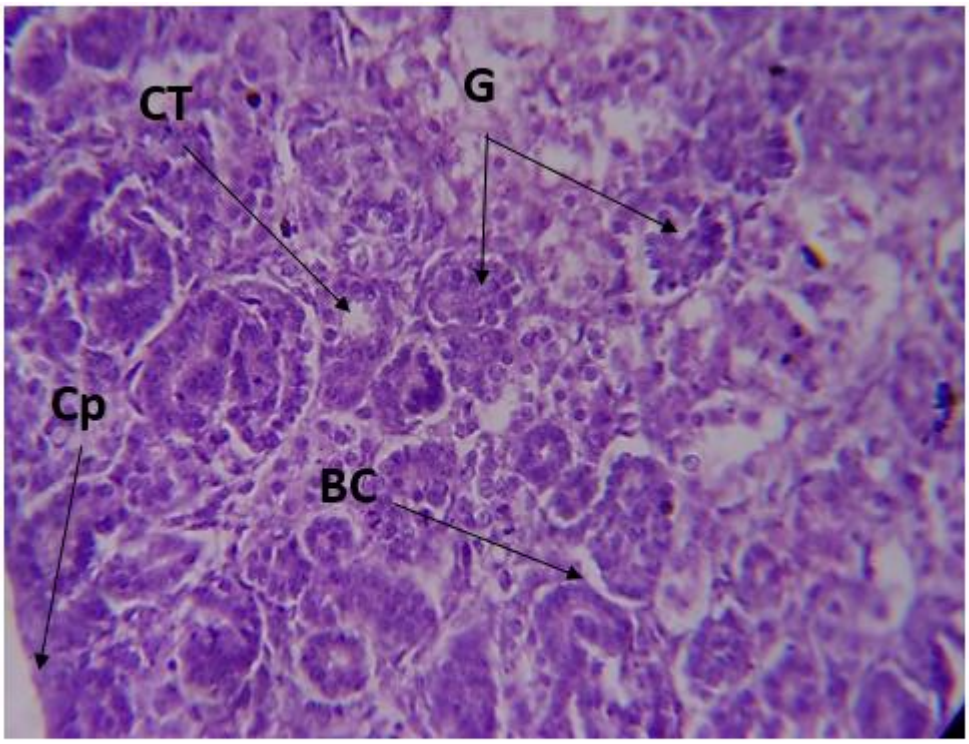

Figure (3): Fetal kidney of probiotic $18*10^6$//0.1 ml group demonstrated the normal formation of glomerulus (G), Bowman's capsule (BC) and convoluted tubules (CT) with capsule (Cp) H&E X400.

The results of the current study also showed that probiotics play an effective role in the development and differentiation of the kidneys faster compared to the control group. According to Flurkey et al. [19], probiotic administration significantly reduces oxidative stress, inflammation, and fibrosis in mice's kidneys, underscoring the crucial functions that probiotics play in anti-oxidant and anti-inflammatory. However, probiotic supplements have been shown to

enhance the activity of antioxidant enzymes, such as catalase, which eliminates reactive oxygen species (ROS) and lessens oxidative damage, and superoxide dismutase, which also eliminates ROS and thereby aids in cell protection [20,21]. The comet assay results demonstrated that rats given Lactobacillus acidophilus had significant reductions in DNA damage in their kidneys, which in turn improves the protection of fetuses from high levels of antioxidants in the mother and thus protects her fetus from any damage or accelerates up cell division and differentiation. Probiotic supplements also support the DNA of cells. According to numerous studies, probiotics enhance intestinal barrier integrity, inhibit the growth of pathogens, and regulate immunological function, all of which are beneficial to health [22, 23]. Probiotics may also improve cellular function in adults, reduce the progression of chronic kidney disease, and partially restore kidney function as measured by glomerular filtration rate [24,25]. This is then mirrored in the developing fetus during pregnancy, as enhanced immune function and increased antioxidant levels in mothers promote cell differentiation and fetal development in all fetal organs, including the kidneys.

Pax2 gene expression

Table (2) demonstrate that gene expression in studied groups, the mean of folding changes in control group was 0.9325. in $9*10^6$/0.1 ml probiotic group folding changes was 0.9667. in $18*10^6$/0.1 ml probiotic group folding changes was 1.021. The results showed non-significant (P=0.001) changes in gene expression between studied groups. In Figure (4) Curves of amplification were the X-axes represent the number of cycles while Y-axes represent in testing Florence. Positive samples showed curve shape of amplification at different cycle number on Roy channel, while negative specimens display know curve amplification at any point of the cycle number and will remain flit blow the threshold level of amplification.

Table (2): The expression level of Pax2 gene in fetal liver groups.

Groups	Pax2	GAPDH	ΔCT	ΔΔCT	2-ΔΔCt	Folding	Mean	P value
Control group	22.1	21.8	0.3	-8	0.92850902	0.92850902	0.932523	1.273
	23	21.9	1.1	-7.2	0.793598721	0.793598721		
	22.9	22.8	0.1	-8.2	0.897097442	0.897097442		
	23.8	21.8	2	-6.3	1.162667732	1.162667732		
	22.9	21.7	1.2	-7.1	0.583220971	0.583220971		
	23.3	21.8	1.5	-6.8	1.230046913	1.230046913		
$9*10^6$/0.1 ml probiotic group	22	22.6	-0.6	-8.9	0.986232704	0.986232704	0.966754	
	21.9	22.1	-0.2	-8.5	0.547424624	0.547424624		
	21.4	22.2	-0.8	-9.1	1.037983885	1.037983885		
	21.4	22.2	-0.8	-9.1	1.198154185	1.198154185		
	22.2	22.9	-0.7	-9	1.057018041	1.057018041		
	22.9	22.1	0.8	-7.5	0.973712312	0.973712312		
$18*10^6$/0.1 ml probiotic group	22.2	22.9	-0.7	-9	1.057018041	1.057018041	1.021414	
	22.9	22.1	0.8	-7.5	0.973712312	0.973712312		
	22.4	22.3	0.1	-8.2	1.097371833	1.097371833		
	23.3	22.1	1.2	-7.1	0.983250971	0.983250971		
	22.8	22.5	0.3	-8	0.858565436	0.858565436		
	22.2	22.3	-0.1	-8.4	1.158565436	1.158565436		

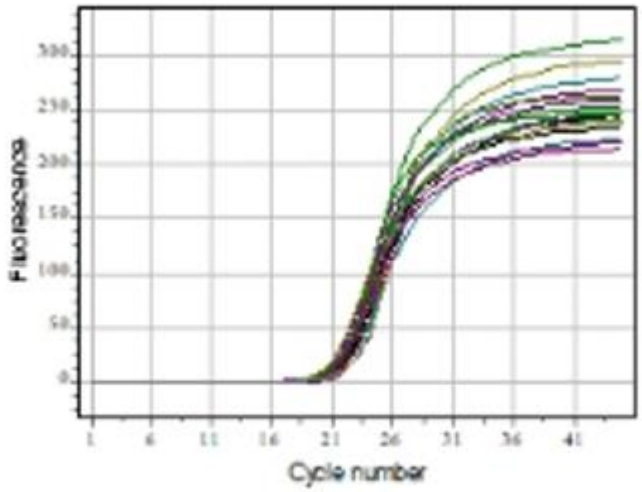

Figure (4): A positive sample with a curve shape at different cycle numbers is displayed by Pax2 gene amplification on the Cy5 channel.

After using probiotic supplements in the current study, there was a slight increase in the expression of the pax2 gene and there was no significant difference compared to the control group. This slight increase may be one of the main reasons for the development of kidneys in the embryos of mice that were injected with probiotics. The mouse genome contains orthologs of the extremely evolutionarily conserved PAX genes. A mouse at the 4–6 somite stage, Pax2 is expressed in the intermediate mesoderm, and at the 12 somite stage, it is found in the nephric duct [26]. The ureteric bud, metanephric mesenchyme, mesonephric tubules, and pronephric duct all still express Pax2. The condensing mesenchyme surrounding the ureteric bud tip exhibits high levels of expression [27]. Pax2 expression is down-regulated during the epithelialization of induced mesenchyme. Mature proximal and distal tubules do not exhibit Pax2. However, throughout maturity, Pax2 is still expressed by the renal collecting ducts, which are derived from the ureteric bud [28]. Herzlinger et al. [29] and Kispert et al. [30] referred that pax2 is one of the earliest markers of mesenchymal induction and may be activated by primary induction signals from the bud. They also indicated that gene expression is responsible for the differentiation of the urinary convoluted tubule lining and visceral cells in the kidney during embryonic stages. This may explain why the slightly higher Pax2 expression in the probiotic-treated groups was the reason for the better kidney differentiation and development compared to the control group in the current study. In the current study, it was found that probiotics have a slight effect on the expression of the Pax2 gene. Previous studies have confirmed that probiotics have an effective role in improving the gene expression of some immune and synthetic genes in some animals indirectly by reducing the effect of oxidative stress in cells, which affects nucleic acids and may reduce gene expression [31,32,33].

Conclusions

The current study concludes that the use of probiotics improves kidney development in fetuses, as the nephrons and urinary convoluted tubules were more developed and differentiated compared to the control group, which demonstrates the effective role of probiotics. Probiotics also led to improved expression of the Pax2 gene in fetal kidneys, which is one of the genes responsible for kidney development during the embryonic stages.

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