ORİJİNAL MAKALE / ORIGINAL ARTICLE

Balıkesir Sağlık Bilimleri Dergisi / BAUN Sağ Bil Derg
Balıkesir Health Sciences Journal / BAUN Health Sci J
ISSN: 2146-9601- e ISSN: 2147-2238
Available online at: https://dergipark.org.tr/tr/pub/balikesirsbd

Does Diabetes Affect the Carbohydrate Secretions of the Endometrial Glands of Rats in Early Pregnancy?

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Geliş Tarihi / Received: *27.01.2020,* ***Kabul Tarihi / Accepted:*** *07.05.2020*

Acknowledgement: This work was supported by Mustafa Kemal University Scientific Research Project Coordination Unit (9761). Some of the laboratory work was completed in Tierärztliche Hochschule Hannover (TiHo Hannover) within the scope of The Scientific And Technological Research Council of Turkey (TÜBİTAK), 2214-A scholarship for doctorate studies. This work is the thesis study of Feyza BAŞAK.

ABSTRACT

Aim: Endometrial gland secretions play an important role in uterine receptivity, and before the implantation they provide nourishment for the embryo. Diabetes is known to alter the secretions of certain cells. This study aimed to identify the carbohydrate content of endometrial gland secretions using Peanut Aglutinin (PNA), Wheat Germ Agglutinin (WGA) Concanavalin-A (Con-A), Soy Bean Agglutinin (SBA).

Materials and Methods: Thirty-two *Wistar Albino* rats were divided into 4 groups of 8; diabetes and pregnancy positive, diabetes positive, pregnancy positive, control group. Samples were collected on the 5th and 7th day of pregnancy, and in the vaginally stimulated non-pregnant rats.

Results: Positive PNA staining occurred in both groups with diabetes and was negative in the other two groups. Diabetes produced marked alterations in SBA staining in the early days of pregnancy, but did not persist for long. With regard to WGA, when diabetes combined with pregnancy, changes in the secretion of the endometrial glands occur. Con-A staining showed that both pregnancy and diabetes affect the amounts of the α-mannose chain, α-chain glucose, and N-acetyl glucosamine α-chain components of endometrial gland secretions.

Conclusion: In conclusion this study shows that both diabetes and pregnancy affect the carbohydrate content of the secretions from the endometrial glands.

Key words: Diabetes; Endometrial Gland; Lectin Histochemistry; Rat; Streptozotocin

Erken Gebelikte Diyabet, Sıçanların Endometrial Bezlerinin Karbonhidrat Salgılarını Etkiler mi?

ÖZ

Amaç: Endometriyal bez salgıları uterus alıcılığında önemli bir rol oynar ve implantasyondan önce embriyonun beslenmesini sağlar. Diyabetin belirli hücrelerin salgılarını değiştirdiği bilinmektedir. Bu çalışmada, Fıstık Aglutinin (PNA), Buğday Germ Aglütinin (WGA) Concanavalin-A (Con-A), Soya Fasulyesi Agglutinin (SBA) kullanılarak endometriyal bez salgılarının karbonhidrat içeriğinin tespit edilmesi amaçlanmıştır.

Gereç Yöntem: Otuz iki Wistar Albino rat, her birinde 8 adet rat bulunan 4 gruba ayrıldı; diyabet ve gebelik pozitif, diyabet pozitif, gebelik pozitif, kontrol grubu. Numuneler gebeliğin 5. ve 7. günlerinde ve gebelik olmayan gruplarda da vajinal olarak stimule edilmiş sıçanlardan stimülasyonu izleyen 5. ve 7. günlerde toplandı.

Bulgular: Her iki diyabetli grupta pozitif PNA boyaması meydana geldi ve diğer iki grupta boyanma olmadı. Diyabet, gebeliğin ilk günlerinde SBA boyamasında belirgin değişikliklere neden oldu, ancak uzun süre devam etmedi. WGA ile ilgili olarak, diyabet gebelikle birleştiğinde, endometriyal bezlerin salgılanmasında değişiklikler meydana geldiği görüldü. Con-A lektini ile yapılan boyamalar hem gebeliğin hem de diyabetin endometriyal bez salgılarının α-mannoz zinciri, α-zincir glikoz ve N-asetil glukozamin α-zincir bileşenlerinin miktarlarını etkilediğini göstermiştir.

Sonuç: Sonuç olarak bu çalışma hem diyabetin hem de gebeliğin endometriyal bezlerden salgıların karbonhidrat içeriğini etkilediğini göstermektedir.

Anahtar Kelimeler: Diyabet, Endometriyal Bez, Lektin Histokimyası, Rat, Streptozotosin.

INTRODUCTION

Over the past decade, knowledge of the pathogenesis and natural history of type 1 diabetes has grown substantially, particularly with regard to disease prediction and heterogeneity, pancreatic pathology, and epidemiology (Stevens et al., 2007). However, despite broad organizational, intellectual, and fiscal investments, no means for preventing or curing type 1 diabetes exists, and, globally, the quality of diabetes management remains uneven (Monsefi et al. 2013). Pregnancy in type 1 diabetic women is associated with an increase in risk both to the fetus and to the mother. Diabetes is one of the most frequent chronic diseases in women of childbearing age, which significantly increases the risk of complications at every stage of pregnancy (Gutaj et al., 2013). Diabetes is a metabolic disease with effects on many systems and is well known to alter the secretions of certain cells (Isola et al., 2012; Lilliu et al., 2012).

Endometrial gland secretions provide nourishment for the embryo in the uterus throughout the implantation process. Even after implantation, it takes some time for the blood circulation between mother and the embryo to become organized, and during this period the embryo continues to be supported by nutrients provided by the endometrial glands. Endometrial secretions are highly glycosylated in both pregnant (Bychkov & Toto, 1986; Bychkov & Toto 1987; Kupryjancyzk, 1989; Lee & Damjanov, 1985) and non-pregnant animals (Jones et al., 1998; Lee & Damjanov, 1985).

Lectins are proteins, or glycoproteins, that specifically bind to sugars both on the cell surface and inside the cell (Öztabak, 2005). And they can be used to characterize the components of endometrial gland's highly glycosylated secretions, since these secretions are important nutritional sources for the developing embryo and contain a variety of growth factors (Burton et al., 2007; Hempstock et al., 2004).

The aim of this study was to investigate the effects of both pregnancy and experimentally induced diabetes on the carbohydrate characteristics of secretion of the endometrial glands, by using the lectin histochemistry method.

MATERIALS AND METHODS

Thirty-two adult female *Wistar albino* rats weighing 250-300 g were used in the study. They were obtained from Mustafa Kemal University Experimental Research on Application and Research Center. Rats were fed *ad libitum* and were maintained in 12-hour light-dark cycles. The rats were divided into 4 groups of 8 rats as follows: Group 1; diabetes induced in pregnancy, Group 2; diabetes induced, Group 3; pregnant, Group 4; control group.

For all procedures the Mustafa Kemal University Animal Experiments Ethics Board guidelines were followed. The female rats were left together with one male rat in the cage overnight. The following day vaginal smears were performed and those rats with spermatozoon in their smears were considered to be in day 1 of pregnancy (Lohmiller and Swing, 2006). Streptozotocin (STZ) (Sigma-Aldrich® S0130, Germany) was administered at a dose of 45 mg/kg (Cornejo-Garrido et al., 2014). intraperitoneally to Group 1 and Group 2 rats. After 2 weeks, animals with blood glucose concentrations greater than 200 mg/dl (Cornejo-Garrido et al., 2014) were assumed to be diabetic. The diabetes was primarily developed and then the rats became pregnant in the diabetes and pregnancy positive group. On the 5th and 7th day of pregnancy (or post vaginal stimulation in non-pregnant groups) the rats were anaesthetized with a Ketamine-Xylazine combination (80-12mg/kg, respectively) and hysterectomy was performed. The vaginal stimulation was performed via a glass rod placed into the vagina and moved backwards and forwards a few times.

The uterine tissues were isolated, fixed in buffered formaldehyde solution [100ml Formalin (37-40% stock solution), 900ml water 4g/L NaH_2PO_4 and 6.5g/L NaH_2PO_4] and then embedded in paraffin blocks from which sections were cut. The 5 µm sections were taken on adhesive slides. In this study, four types of lectins were used; three biotin labeled and one peroxidase labeled. All lectins were obtained from Sigma-Aldrich®. The detail of the lectins is shown in Table 1.

Table 1: Details of the lectins used

Name of the lectin	Nominal Sugar Specificity	Dilution	Catalog number	Source
PNA	β-linked galactose	1:50	L6135	Sigma Aldrich®
SBA	α and β linked N-acetylgalactoseamine	1:50	G2762	Sigma Aldrich®
WGA	β linked N-acetylglucosamine sialic acid	1:50	L5142	Sigma Aldrich®
Con-A	α- linked mannose α- linked glucose α linked N-acetylglucasamine	1:50	61760	Sigma Aldrich®

After deparaffinization, the slides were treated with hydrogen peroxide solution to prevent endogenous peroxidase activity. Then slides were washed with Phosphate Buffered Saline (PBS pH=7.2-7.4) for

rehydration and incubated in serum blocking solution. The lectins were dissolved in PBS. All lectins were diluted 1:50 and incubated with the tissues on the slides for 60 minutes. Slides were then treated with biotin labeled secondary antibodies and incubated with enzyme conjugate and stained with AEC Chromogen Kit (SigmaAldrich®; AEC101) under light microscopy. Slides were counter stained with Mayer's hematoxylin for 10 seconds and mounted with a water-based medium. In the case of peroxidase labeled SBA, the slides were washed with PBS after 60 minutes of lectin application and were treated with AEC Chromogen Kit (SigmaAldrich® AEC101). Then, the same procedure was performed and the samples were prepared for examination under a light microscope. The parotid glands of rats were used as positive control in this study.

RESULTS

For diabetes and pregnancy positive group, in the uterus samples taken on the 5th day, endometrial gland cells stained positive for PNA from the cytoplasm on the apical surface of the cell (Fig 1-A). On the samples from day 7, PNA lectin histochemistry was positive (Fig 1-B). On day 5 endometrial glands showed moderate staining with SBA (Fig 1-C). Moderate intracytoplasmic staining was seen with SBA lectin staining (Fig 1-D) on the day 7 samples. Positive staining was seen with WGA (Fig 1-E) and Con A (Fig 1-G) on samples from day 5 and day 7 (Fig 1-F, H).

For diabetes positive group, in samples from day 5, strong positive staining was seen with PNA lectin (Fig 2-A) and on day 7 samples PNA lectin staining was positive (Fig 2-B). SBA staining results were similar to the results of PNA staining for day 5 (Fig 2-C), but diminished on the 7th day, where only moderate staining was present (Fig 2-D). WGA staining was negative on both days 5 and 7 (Fig 2-E, F). With Con A staining, positivity was seen on day 5 on day 7, whereas lectin staining was negative (Fig 2-G, H).

For pregnancy positive group, in the uterus samples taken on day 5, PNA and WGA lectin staining were negative and the others were positive (Fig 3-A, C, E, G). In samples from day 7, only PNA remained negative and all others lectins had positive reactions (Fig. 3-B, D, F, G).

In control group, for day 5 samples, PNA, SBA and WGA lectin staining was negative (Fig 4-A, C, E), while Con- A lectin staining was mildly positive (Fig 4-G). On the day 7 samples, while PNA and WGA staining was still negative (Fig 4-B, F), Con-A samples showed positive staining (Fig 4-H). SBA lectin staining was also positive (Fig 4-D).

The PNA staining patterns showed that induction of diabetes increased the ß-chain galactose component of endometrial gland secretions on the 5th and 7th days of pregnancy, and this effect is strong in diabetic rats in early pregnancy.

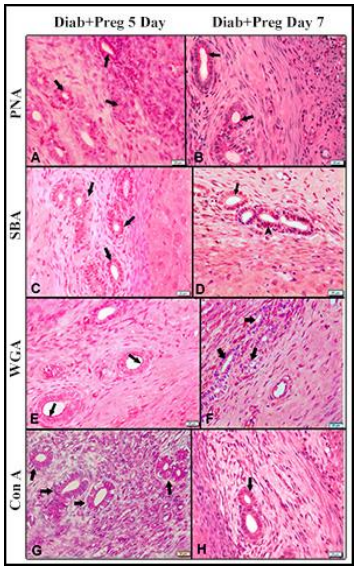

Figure 1. Group 1 lectin histochemistry results. **A)** 5th day PNA, **B)** 7th day PNA, **C)** 5th day SBA, **D)** 7th day SBA, **E)** 5th day WGA, **F)** 7th day WGA, **G)** 5th day Con-A, **H)** 7th day Con-A staining. **arrows:** showing endometrial glands and visible reactions, **arrow head:** showing picnotic nuclei.

Both diabetes and pregnancy influenced the α-ß-chain N-acetylglucosamine components of endometrial gland secretions. Comparing Group 1 with 3, it can be seen that SBA lectin binding to endometrial gland secretions becomes more visible as diabetes induced, i.e. the composition of the secretions changes with regard to the α-ß-chain N-acetylglucosamine components. Comparing Group 2 and 4; it appears that induction of diabetes has a significant effect on SBA lectin binding in early pregnancy, but this effect rapidly diminishes.

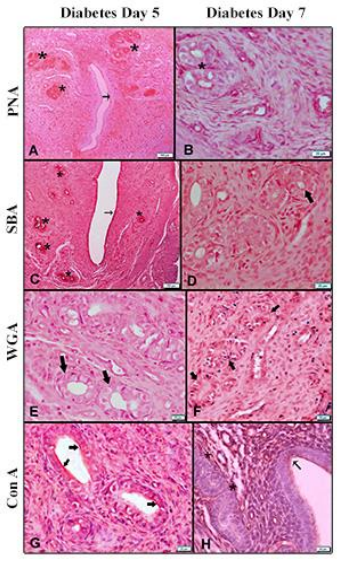

Figure 2. Group 2 lectin histochemistry results. **A)** 5th day PNA, **B)** 7th day PNA, **C**) 5th day SBA (arrow: uterine lümen), **D)** 7th day SBA (arrow: positive staining of the endometrial gland), **E)** 5th day WGA, **F)** 7th day WGA (arrow: negative staining) **G)** 5th day Con-A, **H)** 7th day Con-A staining (asterisk: endometrial gland staining).

For WGA staining in Groups 3 and 4, it is clear that the ß-chain N-acetylglucosamine and sialic acid components are dominant in the early days of

pregnancy. Comparison of Groups 2 and 4 suggests that diabetes itself has no effect on endometrial gland secretions, but when combined with pregnancy (comparing Groups 1 and 4) results in changes in secretion of the ß-chain N-acetylglucosamine and sialic acid components.

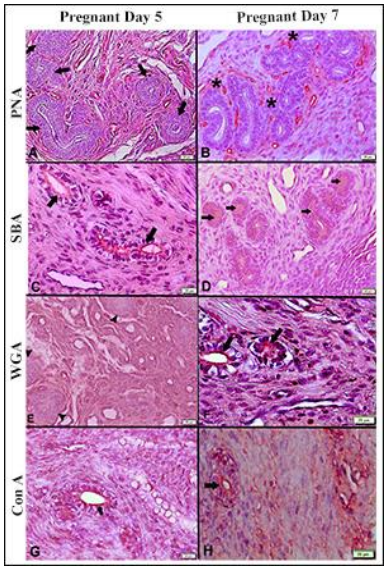

Figure 3. Group 3 lectin histochemistry results. **A)** 5th day PNA, **B)** 7th day PNA, **C**) 5th day SBA, **D)** 7th day SBA, **E)** 5th day WGA, **F)** 7th day WGA, **G)** 5th day Con-A, **H)** 7th day Con-A staining. Arrows, asteriks and arrow heads; degree of staining in the endometrial glands.

Results of Con-A staining, showed that pregnancy and diabetes affected the amounts of α- mannose chain, α-chain glucose, and N-acetyl glucosamine α-chain components of endometrial gland secretions both the 5th and 7th day.

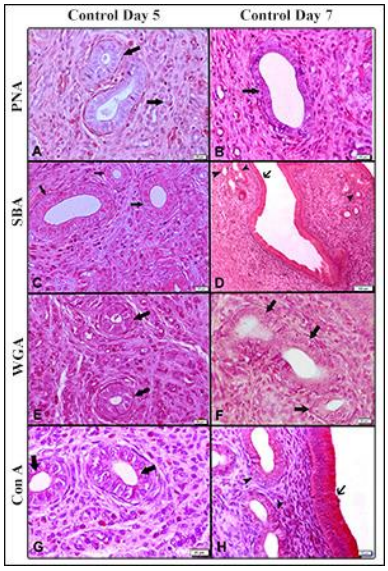

Figure 4. Group 4 lectin histochemistry results. **A)** 5th day PNA, **B)** 7th day PNA, **C)** 5th day SBA, **D)** 7th day SBA, **E)** 5th day WGA, **F)** 7th day WGA, **G)** 5th day Con-A, **H)** 7th day Con-A staining. Arrows and arrow heads: positive staining in the lumen except in Figures D and H. In Figures D and H arrows showing the positive staining in the uterine lumen

The results of the study are summarized in Table 2.

DISCUSSION

There are many reports of the lectin histochemistry of normal and pregnant endometrium in different species (Bychkov & Toto, 1986; Bychkov & Toto 1987; Jones et al., 1998; Jones et al., 2010; Kupryjancyzk, 1989; Leitner et al., 2003; Lee & Damjanov, 1985; Munson et al., 1989; Walter & Bavdek, 1997).

Table 2: Summary of results

Lectins	Group 1		Group 2		Group 3		Group 4	
	5th day	7th day	5th day	7th day	5th day	7th day	5th day	7th day
PNA	+	+	++	+	-	-	-	-
SBA	±	±	++	±	±	+	-	+
WGA	+	+	-	-	-	+	-	-
Con A	+	+	+	-	-	±	±	+

To the best of our knowledge, no studies have shown the lectin histochemistry of endometrial glands in STZ induced diabetic rats. Nor has the lectin histochemistry of endometrial glands from genetically diabetic and congenitally diabetic rats been reported. This is the first report of the composition of endometrial secretions in rats with experimentally induced diabetes. Due to the lack of data for this species, the Group 1 (diabetes and pregnancy positive group) and the Group 2 (diabetes positive) were not compared with other studies. The comparison of these groups was done within the groups of the study.

This study allowed us to identify the difference between endometrial gland secretions in pregnant diabetic, diabetic, pregnant and a control group of rats. In rats, implantation occurs 5th day postcoitus (Dey et al., 2004). 7th day af the pregnancy is the end of first trimester since pregnancy continues for 21 days in rat. In this study implantation and early pregnancy was considered. Altered expression of glyco-conjugates reflect differences in cell differentiation and function (Peel & Bulmer, 1996).

In studies of rats and mice (Akif et al, 1995; Monsefi et al, 2013; Peel & Bulmer, 1996; Stewart et al., 2000) none of the control or pregnant groups showed positive PNA lectin staining. The findings of this study are in accordance with previous studies in this respect. However, in the diabetic animals (Group 1 and 2) in our study, positive PNA lectin staining was seen. Results of this study demonstrate a significant difference between the diabetic and non-diabetic groups with respect to PNA lectin staining on both

days 5 and 7. We speculate that diabetes and diabetes combined with pregnancy affect PNA lectin concentrations in endometrial gland secretions.

For SBA lectin, there was strong staining in diabetic animals (Group 2) on day 5 compared to the control group but this staining was only faintly visible on day 7, and the results from Group 1 were different from all other groups. We conclude that pregnancy and diabetes alone and diabetes combined with pregnancy change the composition of the endometrial gland secretions with regards to SBA lectin. This means the α-ß chain N-acetylglucosamine components of the endometrial gland secretions are significantly affected by both pregnancy and diabetes.

Significance differences were seen in WGA lectin staining with pregnancy. The negative staining in diabetic rats became positive in the diabetic pregnant rats. Since the control group also stained negative for this lectin, we conclude that diabetes combined with pregnancy alters the uterine secretions.

Con-A results were the same for diabetic animals whether or not they were pregnant, but were different from the control groups. This suggests that the secretion of Con-A specific carbohydrates is also affected by pregnancy and diabetes alone and diabetes combined with pregnancy.

Peel and Bulmer (1996) examined rats on the 10th, 12th and 15th day of the pregnancy, and found positive staining for Con- A and WGA in endometrial glands. In group 3, (pregnancy positive group) we found positive staining for Con-A lectin on both day 5 and 7. But WGA staining was negative on day 5 and positive on day 7. In the same study (Peel and Bulmer, 1996) staining for SBA and PNA was negative in the endometrial glands. In this study, PNA staining was negative in rat endometrial tissue samples obtained on the 5th and 7th days of pregnancy. So PNA specific carbohydrate ß-chain galactose was seen not to contribute to the endometrial gland secretions until the end of the first trimester. The findings of Peel and Bulmer (1996), suggest that this carbohydrate type was not found in secretions till the end of second trimester. We found no SBA lectin staining on day 5 but staining increased on day 7 of pregnancy (Group 4), unlike Peel and Bulmer (1996), who found no staining on 10th, 12th and 15th day of pregnancy. This may suggest that the carbohydrate content of endometrial secretions alters as the embryo develops, and secretions only contains α-ß-chain N- acetylglucosamine components in the early stages of pregnancy. The endometrial gland secretions appear to alter according to the needs of the embryo.

In a study performed on the ovarian and endometrial tissues of non-pregnant rats (Lutsky & Sogomonian, 2012) secretions were positive for SBA and negative for the PNA, WGA and Con-A lectins. The control group in this study confirms these results with the exception of Con-A lectin, for which we detected positive staining seven days after vaginal stimulation. Vaginal stimulation was performed in non-pregnant rats in an attempt to make experimental conditions similar in all groups but it is possible that the difference between this study and findings of Lutsky and Sogomonian's (2012) could be due to the use of vaginal stimulation. Further studies are needed to determine if this was a factor in the results.

In another study (Akif et al., 1995) lectin histochemistry staining methods were used to show the effects of sexual cycles on mouse endometrium. In that study no PNA lectin staining was detected in endometrial tissues in any of the cycles - a similar finding to the control group in the present study. In addition Akif et al., (1995) showed negative SBA lectin staining in proestrus, estrus, and diestrus periods, but positive staining was found in metestrus. In our study, SBA lectin staining was negative on day 5 and positive on day 7. The staining variations among control group rats might be caused by them being in different stages of the sexual cycle. Further studies would be required to look at the effects of species and cycle- dependent changes.

In conclusion, these results show that both pregnancy and diabetes affect the lectin histochemistry of the endometrial glands; namely, the type and the amount of carbohydrate components secreted alters due to pregnancy and diabetes.

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