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Glycogen Changes in the Rat Uterus During Different Phases of the Oestrous Cycle

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ABSTRACT

Objective: to study the glycogen content in the rat uterus at oestrous cycle (oes).
Design: an experimental study of the glycogen content in the rat uterus at (oes).
Setting: done during the period of 2000-2002 in the department of anatomy Mosul Medical College.
Methods: this study was carried out on 24 sexually mature virgin female rats of 13-15 weeks old and 165-270 gram weight. They were divided into four groups according to the phases in oestrus cycle 1. Metoestrus 2.dioestrus 3. Proestrus and 4. Oestrus phases, the uterine horns were divided into pieces of 3-4mm and prepared for histological examination, then sections of six micron thickness were collected and stained by alcian blue-periodic acid Schiffs' reagent (PAS). The glycogen was shown as magenta color.
Result: cellular proliferation was noticed at dioestrus and proestrus phases then followed by an increase in the glycogen content at proestrus which became highest at oestrus phase, which is mainly concentrated in the endometrium.
Conclusion: this study showed increase in glycogen content at proestrus and oestrus phases mainly concentrated at the endomatrium, due to consumption of glycogen by the future nidating zygot after mating and fertilization which occur in the endometrium later.
Keywords: Glycogen, rat, utrus, oestrus cycle.

دراسة التغيرات الحاصلة في الكلايكوجين لارحام الجرذان خلال الاطوار المختلفة من الدورة الودقية

الملخص

الهـدف: لدراسة التغيرات الحاصلة في الكلايكوجين لارحام الجرذان خلال الاطوار المختلفـة مـن الـدورة الودقية.

التصميم: دراسة تجريبية للكلايكوجين في ارحام الجرذان على فترات مختلفة من الدورة الودقية.

تاريخ ومكان البحث: اجري البحث في عام 2000-2002 في قسم التشريح-كلية الطب-جامعة الموصل.

الطريقة: اجريت الدراسة على 24 من اناث الجرذان البالغات جنسيا والباكرات يتـراوح عمرهـا 13-15 اسبوعا ووزنها 165-270 غرام وقد قسمت الى 4مجاميع بحثية حسب الاطوار الودقية 1. الطور الذي يلي

الودق 2.طور الاستكنان الودقي 3.الطور الذي يسبق الودق 4.طور الودق نفسه، وبعد جمع قرون الارحـام وتقطيعها بحجم 3-4 ملم قطع منها شرائح نسيجية بـسمك6مـايكرون وصـبغت بAlcian blue(PAS) وظهر الكلايكوجين بلون بنفسجي.

النتائج: لوحظ زيادة الانقسامات الخلوية في طور الاستكنان الودقي والطور الذي يسبق الودق تلاها زيادة في الكلايكوجين خلال الطور الذي يسبق الودق حيث وصل ذروته في طور الودق نفسه وهذه الزيادات متركزة بصورة رئيسية في الغشاء المبطن للرحم يلي ذلك في عضل الرحم وبصورة اقل في ظهارة الرحم الخارجية.

الاستنتاج: لوحظ في هذه الدراسة ان الكلايكوجين يزداد في الطور الذي يسبق الودق ويصل ذروته في طور الودق نفسه ويتركز في الغشاء المبطن للرحم لان الكلايكوجين يعتبر مادة غذائية مهمة عند انزراع البويضة المخصبة بعد الجماع والاخصاب والذي يتم بالتحديد في الغشاء المبطن للرحم.

INTRODUCTION

The glycogen content in the uterine tissue was detected by using Alcian blue-PAS reagent (Pears, 1977). The polymerized carbohydrates with a single structure unit were classified into glycogen, starch and cellulose. The presence of large quantity of glycogen in uterine cells represent an increase in the activity of these cells, and also indicates that there is a metabolic activities in the endometrium of the female rat uteri. It has been found that there was a similtanous increase in the collagen fibers parallel to the increase in the glycogen content (Mozanska, 1972, Zamojska, 1990). The decrease in the amount of glycogen reflects the gradual decline in the activity of these tissues which reflects a degenerative process in the uterine endometrium. Other workers stated that the increase in the glycogen content occurred as a result of the transformation of some acid mucopolysaccharide (AMP) to glycogen due to the effect of some cofactors (Dubey, 1991). These physiological chains of cyclical changes in the glycogen content are attributed to hormonal factors, and they are preceded by cellular proliferation and increase in cellular mitotic activity which enhance the process of fertilization after mating to allows spermatozoa to enter the female host and get implanted their (Marcus, 1974).

MATERIALS AND METHODS

Twenty-four virgin female rats of sprague-dawly strain were used. They were 13-15 weeks of age and of 165–270 gm. weight. All were kept in animal house and exposed to day light. They are from departmental stock and maintained on standard diet (which is used in our anatomy department and contain various food material). Vaginal smears were taken each morning and only animals showing two successive normal cycles were used. In the sexually mature rats, the various phases of the oestrous cycle were determined by microscopic examination of unstained lower vaginal smears (Aesten, 1990).The oestrus phase recurs every 4-5 days in the absence of mating six animals were used for each phase of the oestrous cycle.

The rats were sacrificed at morning after the vaginal smear had confirmed the exact phase of the cycle. Hysterectomy was done through a mid abdominal incision using ether for anaesthesia. The two uterine horns were separated and cut into slices of 3-4 mm thickness and fixed in Lavdowsky mixture (Humason, 1972), then, the tissues were

dehydrated in upgraded alcohol, then cleared by two changes of xylol and emersed in three changes of paraffin and finally embeded in paraffin at 60 ^{0}C melting point. Sections of 6 micron thickness were cut from each paraffin block using Richerts rotary microtome then stained by alcian blue-PAS combination to detect glycogen as magenta colour.

RESULTS

The endometrium consists of lining epithelial cells, endometrial stroma, and endometrial glands (the PAS reaction gave magenta colour which indicates glycogen content). The glycogen content within the rat uterus were increased during the proestrus phase and reach its peak at oestrus phase and it was mainly seen in the endometrium, less marked in the myometrium and least in perimetrium (Fig 3 and 4). While in the metoestrus phase the reaction starts to decline and become very weak in the diestrus phase (Fig 1 and 2). These changes in the intensity of PAS reaction were preceded by proliferation of the uterine tissue mainly in the endometrium including its stromal layer. The uterine glands and outer aspect of blood vessels showed the same changes of endometrium. (See Table 1).

Table 1: glycogen content shown as (PAS) positive reaction in different parts of the uterus during the oestrous cycle.

Phases of the Oestrous Cycle	Endometrium				Myometrium			Perimetrium	
	Luminal Epitheliu m	Endometri al stroma	Blood Vessels	Uterine Glands	Circular Layer	Vascular Zone	Longitud . Layer	Conn. Tissue	Mesothel. Cells
1.Metoestrus	–	±	–	±	±	±	–	–	–
2.Dioestrus	–	±	–	–	–	–	–	–	–
3.Proestrus	±	++	+	++	±	±	±	±	±
4 .Oestrous	+	+++	++	+++	+	+	+	+	±

__: negative reaction,±:- very weak positive reaction; + weak positive reaction ++ : moderate positive reaction, +++: strong positive reaction.

DISCUSSION

Positive PAS reaction indicates the presence of glycogen in different parts of the uterus. In this work we noticed that the glycogen starts to increase in proestrus and reachs its peak at oestrus stage of the cycle, preceded by an increase in cellular proliferation in the dioestrus and proestrous phase, mainly concentrated in the endometrium and to a lesser extent in the myometrium and least in the perimetrium.These changes were attributed to increase oesrogen which usually occurs in the midcycle (dioestrus phase), and when the progesteron starts to elevate at proestrus phase the effect becomes more evident due to synergistic action of progesteron and oestrogen, which result into stimulation of their receptors (oestrogen and progesterone receptors) in the uterus (Vuhai, 1978). The increased mitotic activity within the uterus may be oestrogen dependent since oestrogen levels begin to rise during dioestrus, while progesterone level remains very low through proestrus (Butcher, 1974),and the oestrogen stimulates the endometrial mitotic activity (Ross, 1981), due to the presence of increased number of endometrial oestrogen receptors which suggests a comparable ovarian role.

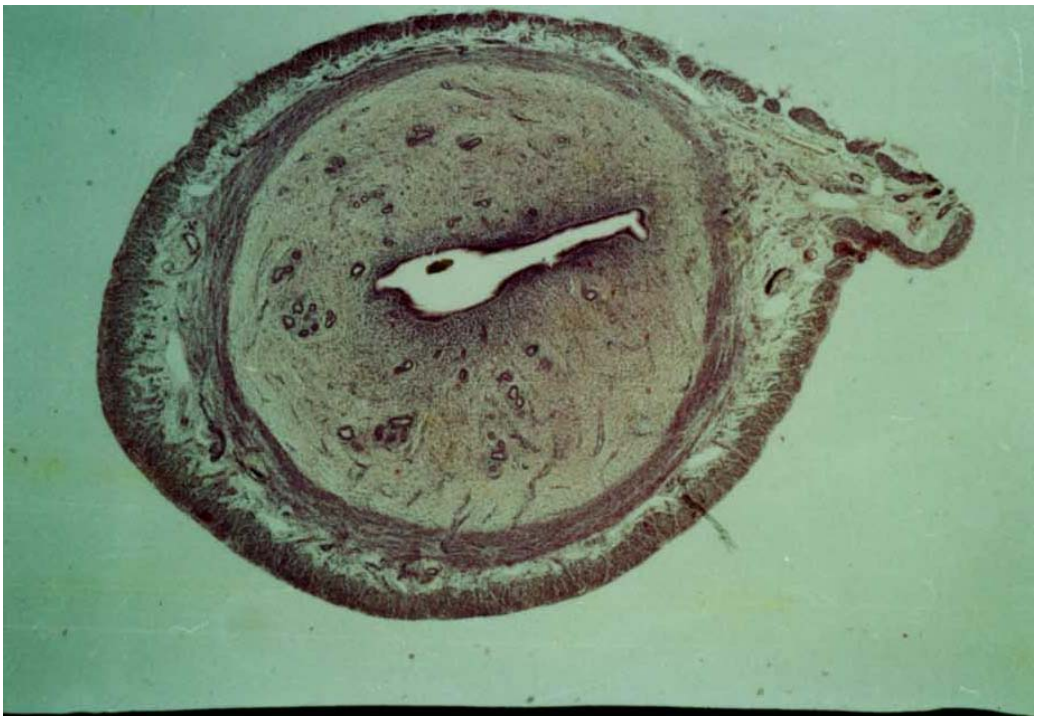

Fig. 1: Transverse section in the rat uterus showing endometrium (e), myometrium (m) and perimetrium (p) at metoestrus phase. Weak PAS reaction in the endometrial stroma, blood vessels and glands. (arrows) (Alcian blue PAS Stain, X30).

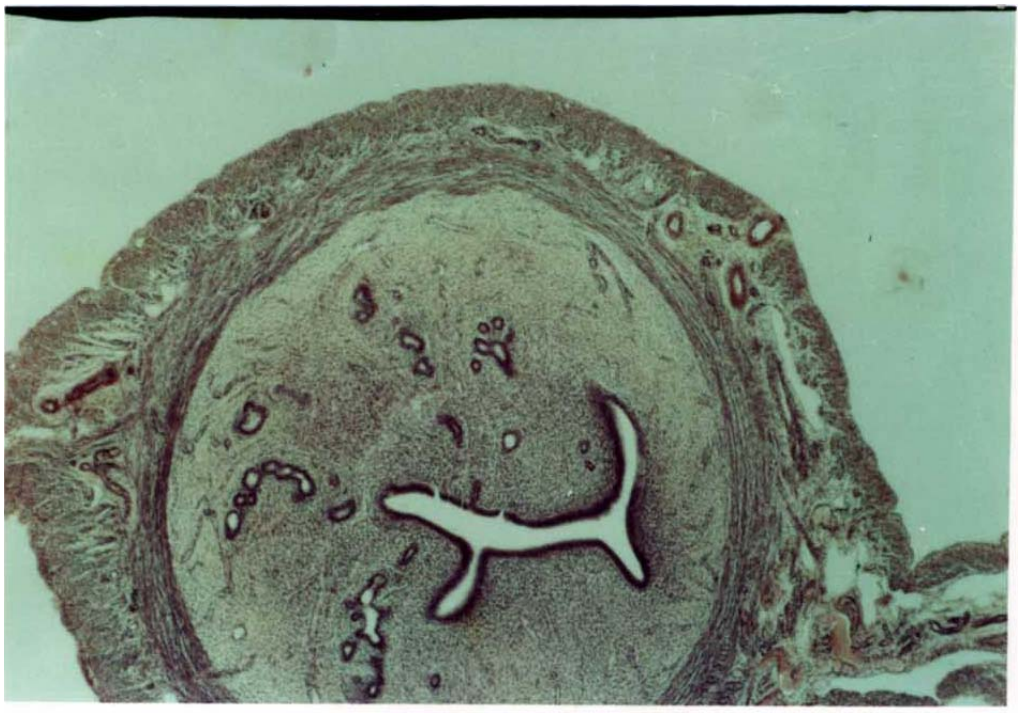

Fig. 2: Transverse section in the rat uterus at dioestrus phase, showing increased cell proliferation and weak PAS reaction in the endometrial stroma.(arrows) (Alcian blue PAS Stain, X60)

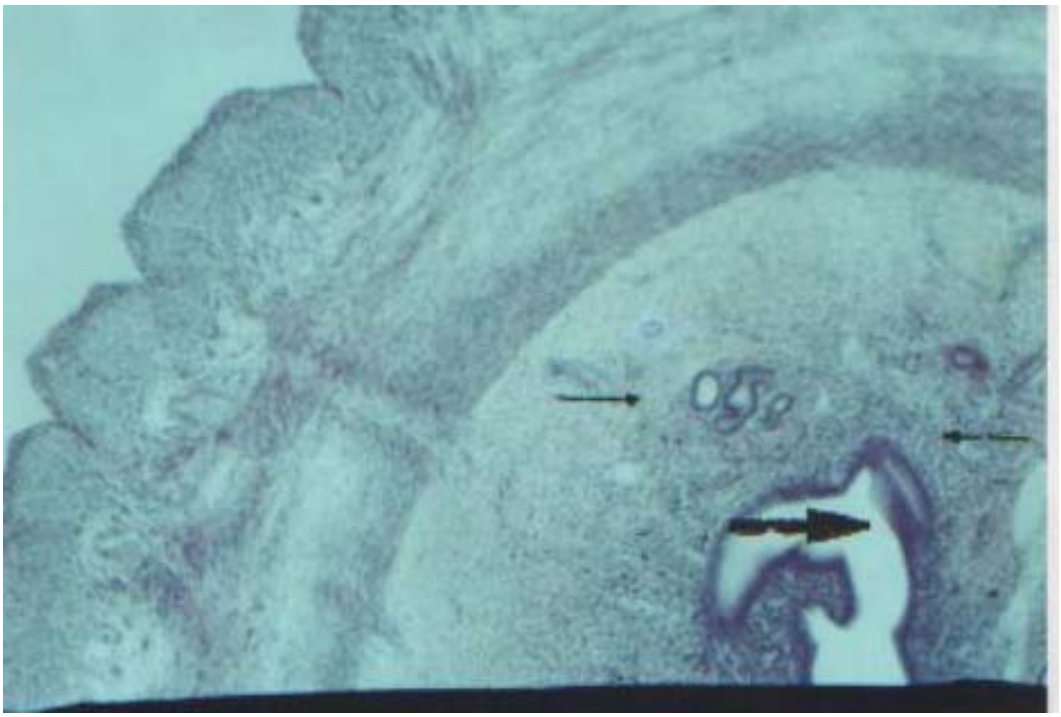

Fig. 3: Transverse section in the rat uterus at the proestrus phase showing PAS positive reaction in the epithelial cells, endometrial stroma, and uterine glands. (arrows) (Alcian Blue PAS Stain, X90).

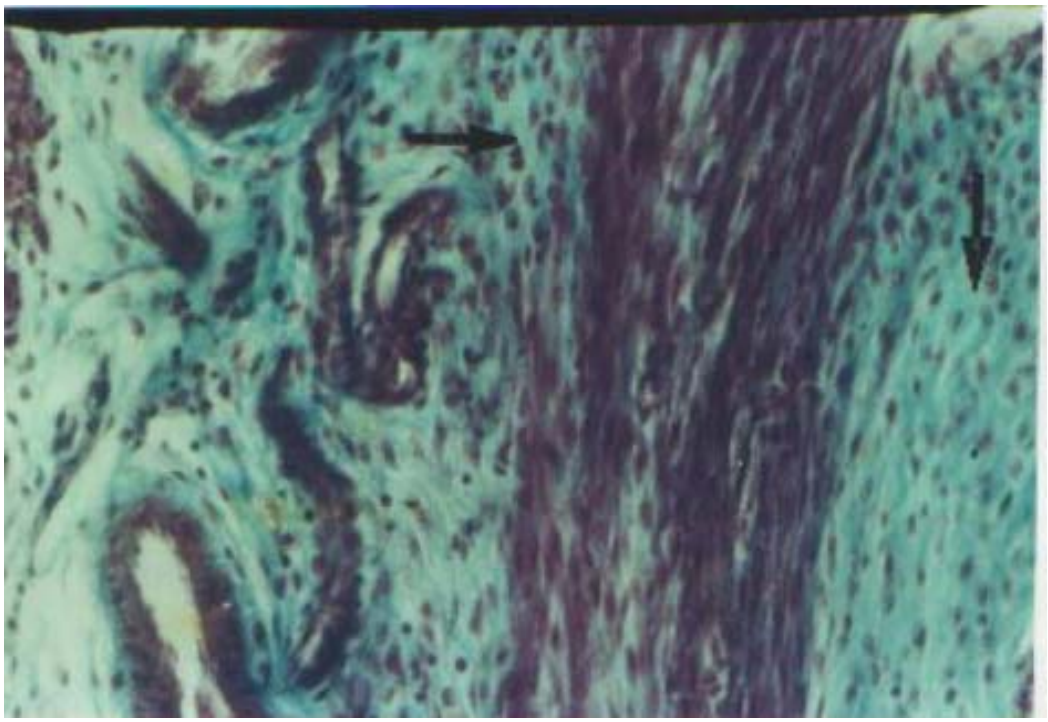

Fig. 4: Transverse section in the rat uterus at oestrus phase showing strong positive PAS reaction in the endometrium, and myomertium. (arrows) (Alcian blue PAS Stain, X180).

The increase in uterine cellular proliferation in diostrus phase of the stromal layer was expected since increased glycogen occurs as a result of the ovarian hormons. Mating occurs only in the oestrus phase of the cycle, the increased uterine cellular proliferation may be interpreted as physiological preparation for the fertilization and implantation in the uterine tissue. It would therefore be reasonable to propose that ovarian hormones, which prepare the female reproductive tract for pregnancy, also prepare the host for the process of mating and implantation. Stevens and Lowe 1997 noticed that the glycogen content is maximal at the time of ovulation in the vagina and some glycogen rich surface squames are shed into the viginal cavity after ovulation. Other workers noticed that glycogen was present in moderate quantity in oestrus stage which decreased in the preimplantation period and starts to disappear during pregnancy, this seems to support our finding, since there was minimum amount of glycogen at metoestrus and dioestrus, and being considered as a preimplantation period for the next cycle, and may be due to inadequate progesterone level, which result in a declined in the thickness of endometrium (Schnurrbasch, 1990), while others noticed a sharp decline in glycogen after mating (Rajalakshmi, 1972), this support our finding in the metoestrus phase, while other workers noticed that glycogen was concentrated in the circular muscles of rabbit uterus as well as in its vascular component, and also increased in the mesometrial site where the implantation usually takes place and they stated that administration of oestrogen to immature castrated rabbits and rats causes elevation of the glycogen both in the endometrial and myometrial layers, while administration of progesterone alone or with either oestrogen or cortisone caused decrease of the glycogen content (Gregory, 1970).

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