A CORRELATIVE STUDY OF ENDOMETRIAL GLYCOGEN CONTENT AND OTHER CONTRIBUTORY FACTORS ON FEMALE INFERTILITY

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Abstract

Background and Objective: Endometrial glycogen is one of the most important factors for development of the blastocyst in the early stages of gestation which is reconverted into a simple monosaccharide like glucose at the time of implantation. Hence in the present study, the role of glycogen content in the endometrium and other factors like age, duration of infertility pattern of menstruation and other clinical features in causing infertility was studied.

Materials and Methods: This study was carried out in the Department of Pathology, Bangalore Medical College after the ethical clearance from the institutional ethics committee. The study included 90 cases with complaints of infertility (primary / secondary). The endometrial tissue was fixed; processed, sectioned and performed Periodic Acid Schiff (PAS) stain for detecting the amount of glycogen in endometrium. The present prospective study was a descriptive study and the values are mentioned in percentages.

Result: We observed that, the majority of primary infertility patients were in the age group of 21-25 years and that of secondary were in 26-30 years. Most of the patients 55(74.3%) in primary & 12(75%) had regular cycles. The duration of primary infertility group was 2-3 years and that of secondary infertility group was 4-5 years. The glycogen content in the endometrium was grossly reduced in patients with Luteal Phase Defects.

Conclusion: We found that the glycogen content of endometrium was very much deficient in late secretory phase might be one of the major causes for female sterility.

Keywords: Endometrial Glycogen; Luteal Phase Defect; Primary Infertility; Secondary Infertility

1. Introduction

Infertility is a global public health concern, partly due to its complexity and difficulty in preventing, diagnosing and treating it. More than 80 million people about 8-12% of all couples worldwide are or have been infertile. The prevalence of infertility does not differ significantly among racial and ethnic groups. Although patients seeking treatment for infertility are predominantly of high socioeconomic status, infertility is more common among groups of relatively low socioeconomic status. Improved familiarity with and access to infertility services among the affluent and better-educated patients probably accounts for their greater use of these medical resources¹.

Infertility is the failure to conceive (regardless of cause) after 1 year of unprotected intercourse. Infertility affects approximately 10-15% of reproductive-aged couples. Its overall prevalence has been stable during the past 50 years; however, a shift in etiology and patient age has occurred. As a woman's age increases, the incidence of infertility also increases.

According to American Fertility Society “a marriage is to be considered barren or infertile when pregnancy has not occurred after coitus without contraception”. The most accepted definition is “a couple is considered clinically infertile only when pregnancy has not occurred after at least 12 months of regular sexual activity without the use of contraceptives”². Infertility implies apparent failure of a couple to conceive after one year of “protected” and regular intercourse³. Infertility denotes lack of fertility, an involuntary reduction in the ability to reproduce children⁴. Infertility is defined as the failure of a couple of reproductive age to conceive after 12 months of regular coitus without contraception⁵. The chemical definition of infertility is “the inability of a couple to achieve chemically/biochemically (HCG) recognizable pregnancy after 12 months of regular intercourse⁶.
Endometrial glycogen is one of the most important factors for development of the blastocyst in the early stages of gestation. The reduction of glycogen content in the endometrium is called “glycogen uteri”. McKay et al (1956) have established a definite cyclic pattern for most of the metabolic constituents in the normal endometrium. Glycogen makes its appearance in the glandular epithelium shortly before ovulation and increases progressively in quantity during the secretory phase of the endometrium. It shifts into the lumina of the glands along with the secretion. It is also present in the secretion escaping from the surface of the endometrium immediately before menstruation. There is a slight reduction in the amount of glycogen in the epithelia of the glands at this time. These fluctuations are not seen in the basal layer of endometrium. The glucose circulating in the blood is converted into glycogen and stored in the glands as glycogen which represents the most convenient and readily utilizable form of storage. At the time of implantation this is reconverted into a simple monosaccharide like glucose that could serve as an excellent nutritional basis for the blastocyst, before its actual implantation in the endometrium. Since there was a scarcity in the literature on the role of glycogen content in the endometrium as a determinant of infertility in females, prompted us to undertake this study to explore the role of glycogen content in the endometrium and other factors like age, duration of infertility pattern of menstruation and other clinical features in causing infertility.

2. Materials and Methods:
The present prospective, correlative study was conducted in the Department of Pathology, Bangalore Medical College, Bangalore after the ethical clearance from the institutional ethics committee. The study included 90 cases with complaints of infertility (primary / secondary) who were referred to Pathology Department, from Gynecology Departments of Vani Vilas Hospital, Bowring & Lady Curzon Hospital and other hospitals attached to Bangalore Medical College. The patients who failed to conceive after one year of unprotected coitus following marriage were investigated as cases of primary infertility and the patients who failed to conceive after having achieved a previous conception were investigated as cases of secondary infertility. The patients with complaints of infertility (Primary / Secondary) are included in the present study whereas, those with complaints of infertility within one year of marriage, non co-operative patients and Male factors causing infertility are seen (Semen analysis – abnormal) are excluded from the study. Before examining the case, their husband’s semen analysis was done routinely to rule out the male factors causing infertility. The case was included for the study only if it was a normal semen analysis. Detailed clinical history in the form of menstrual cycle, Last menstrual period (LMP), age at marriage and obstetric history (in secondary infertility) were obtained. The procedure of Dilatation & Curettage (D&C) was explained and informed consent was taken from the entire patient. The premenstrual D & C was done to obtain endometrial biopsy. D & C was done any time in cases of Amenorrhea and prolonged bleeding. The endometrial tissue was fixed in 10% formalin for 24 hours and routinely processed. 5-6 micron sections were cut and performed Periodic Acid Schiff (PAS) stain for detecting the amount of glycogen in endometrium. Age of the patient, duration of infertility, pattern of menstruation and other clinical features was also recorded. The present prospective study was a descriptive study and the values are mentioned in percentages.

3. Result:
The present study reports the role of glycogen content in the endometrium and other factors like age, duration of infertility, pattern of menstruation and other clinical features in causing infertility was recorded.

3.1 Age incidence: The youngest patient seen was 18 years old and eldest was 38 years in primary infertility cases with an average age of 24.2 ± 2.0 years. The youngest patient seen was 25 years old & eldest was 36 years in secondary infertility cases with an average age of 29.75 ± 2.0 years. In primary infertility group maximum cases 43 (58.2%) belonged to the age group of 21-25 years. In secondary infertility group maximum cases 11 (68.7%) belonged to the age group of 26-30 years (Table-1).

3.2 Duration of infertility: In primary infertility group, duration of infertility was calculated from the date of marriage. In secondary infertility group, it was calculated
from the date of last delivery or from the date of last abortion in cases with history of abortion. It was observed that duration of infertility varied with a wide range of 1-13 years. In primary infertility group maximum patients i.e., 35 (47.3%) came within 2-3 years duration of marriage. In secondary infertility group, maximum patients i.e., 6(37.5%) came within 4-5 years of duration of infertility (Table-2).

3.3 Menstrual pattern: The menstrual history was elicited in all patients of primary and secondary infertility. The menstrual history includes age at menarche, past menstrual cycles and specifically the date of last menstrual period. The pattern of menstrual cycle was shown in Table-3.

3.4 Clinical features: The patients came with history of menstrual problems, leucorrhoea, Dysfunctional uterine bleeding & anemia. Majority of patients were asymptomatic, one of the diagnosed TB endometritis patient was also asymptomatic. The distribution of patients with different clinical features was depicted in Table-4.

3.5 Glycogen content of endometrium: Glycogen content of endometrium was studied in 50 cases with 30 cases of infertility and 20 cases of proved fertility which formed control for the study. The premenstrual biopsy was taken in the control group also and histopathology revealed 2 cases of proliferative phase and 18 cases of secretory phase. The study includes 24 cases of primary infertility and 6 cases of secondary infertility. Over all there were 10 cases of proliferative phase, 10 cases of normal secretory phase and 10 cases of luteal phase defect. In the control group showed 2 cases of proliferative phase and 18 cases of secretory phase. The cases showing endometrial hyperplasia, TB endometritis, and polyp were excluded from this study. PAS staining was done in all the cases and graded the glycogen content (Table-5) as follows.

a) Mild (+): Small amount of glycogen in early and mid proliferative phase, distribution is perinuclear and particles are small. b) Moderate (++): In early secretory phase glycogen particles are initially infranuclear & later supranuclear. c) Heavy (+++): In late secretory phase large amount of glycogen in lumen of glands. d) Intense (++++): predecidual cells and stroma also show large masses of glycogen.

Histologically, the endometrium showed mild glycogen in 20%, moderate 35%, heavy 20% & intense 25% in infertility group in secretory phase as compared to control it was mild in nil, moderate in 5%, heavy 17% & intense 78% cases. The amount of glycogen in proliferative phase in both fertile and infertile groups was graded as mild in all cases. Out of 10 cases in infertile group 9 showed mild and one case showed moderate glycogen. In the control group (fertile patients) 2 cases showed mild glycogen (Table-6). This indicates that there is very little glycogen in endometrium during proliferative phase.

Table 1: Age distribution among primary and secondary infertility patients. Total number of cases was 100

| Age in years | Primary | Secondary |
|--------------|---------|-----------|
|              | Number  | Percentage| Number | Percentage |
| 18-20        | 7       | 9.4       | -      | -          |
| 21-25        | 43      | 58.2      | 1      | 6.3        |
| 26-30        | 19      | 25.7      | 11     | 68.7       |
| 31-35        | 4       | 5.4       | 3      | 18.7       |
| 36-40        | 1       | 1.3       | 1      | 6.3        |
| Total        | 74      | 100.0     | 16     | 100.0      |

Inference: Majority of primary infertility patients were in the age group of 21-25 years and that of secondary were in 26-30 years.
Table 2: Distribution of cases according to duration of infertility. Total number of cases was 100.

| Duration of infertility (years) | Primary | Secondary | Total |
|---------------------------------|---------|-----------|-------|
| < 2                             | 3       | -         | 3     |
| 2-3                             | 35      | 4         | 39    |
| 4-5                             | 20      | 6         | 26    |
| 6-7                             | 7       | 1         | 8     |
| 8-9                             | 6       | 3         | 9     |
| 10-11                           | 2       | 1         | 3     |
| > 12                            | 1       | 1         | 2     |
| TOTAL                           | 74      | 16        | 90    |

Inference: Majority of patients in primary group had duration of infertility of 2-3 years & that of secondary group had duration of infertility of 4-5 years.

Table 3: Pattern of menstrual cycle in primary and secondary infertility. Total number of cases was 100.

| Menstrual pattern | Primary infertility | Secondary infertility |
|-------------------|---------------------|-----------------------|
| Regular           | 55                  | 12                    |
| Irregular         | 11                  | 2                     |
| Menorrhagia       | 6                   | 1                     |
| Secondary amenorrhea | 1                 | -                     |
| Polymenorrhagia   | 1                   | 1                     |
| Total             | 74                  | 16                    |

Inference: Majority of patients 55(74.3%) in primary & 12(75%) had regular cycles.

Table 4: Distribution of clinical features in infertility cases. Total number of cases was 100.

| Symptom                   | Number of cases | Percentage |
|---------------------------|-----------------|------------|
| Asymptomatic              | 61              | 67.77      |
| Menstrual problems        | 23              | 25.55      |
| Leucorrhoea               | 1               | 1.11       |
| DUB                       | 2               | 2.22       |
| Anemia                    | 1               | 1.11       |
| Hypothyroidism            | 2               | 2.22       |
| Total                     | 90              | 100        |

Inference: Major complaints of infertility patients were menstrual problems.

Table 5: Glycogen content in different groups of infertile patients. Total number of cases was 30.

| Amount of glycogen | Proliferative phase (10) | Normal secretory phase(10) | Luteal phase defect (10) |
|--------------------|---------------------------|----------------------------|--------------------------|
|                    | primary | secondary | primary | secondary | primary | secondary |
| Mild(+)            | 7       | 2         | -       | -         | 3       | 1         |
| Moderate(++)       | 1       | -         | 1       | -         | 5       | 1         |
| Heavy(+++)         | -       | -         | 3       | 1         | -       | -         |
| Intense(++++)      | -       | -         | 4       | 1         | -       | -         |

Inference: Glycogen was grossly reduced in patients with LPD.
Table 6: Grading of glycogen in secretory phase in fertile and infertile groups.

| Amount of glycogen | Infertile group | Fertile group |
|--------------------|-----------------|---------------|
|                    | 20 cases        | 18 cases      | %      | %      |
| Mild(+)            | 4               | -             | 20     | -      |
| Moderate(++)       | 7               | 1             | 35     | 5      |
| Heavy(++)          | 4               | 3             | 20     | 17     |
| Intense(++++)      | 5               | 14            | 25     | 78     |

Inference: % of ++++ grade in infertile group is very low compared to the same in fertile group.

Fig-1: Age distribution among Primary and Secondary infertility. Total number of cases was 100.

4. Discussion:
The findings of our study are correlating with Zawar. The maximum number of patients approached during 21-30 years. The youngest patient was 20 years old and oldest was 35 years. Majority patients of primary infertility 66.26% belonged to the age 21-25 years and that of secondary infertility belong to the age of 26-30 years\textsuperscript{10, 11}. In our study majority of primary infertility i.e. 43 (58.2) belong to 21-25 years and that of secondary infertility 11 (68.7%) belong to the age group of 26-30 years. The abnormal menstrual patterns included were irregular periods, menorrhagia, polymenorrhagia & amenorrhea.

4.1 Glycogen content in the endometrium: Hughes in 1967 reported that glycogen is present in highest concentration around the 17 to 20 the day of the cycle\textsuperscript{12}. Hong Yul Choi et al. reported that secretory substance in the epithelial cells of the endometrial glands during the secretory phase and menstrual phase was mainly glycogen and concluded that PAS staining is superior to routine hematoxylin and eosin staining for the detection of epithelial secretory substance\textsuperscript{13}. Baveja et al observed that the proper embedding of the ovum depends much on the production of sufficient amount of glycogen by the endometrium. Failure to do so will lead to the development of poor quality of endometrium resulting in death of ovum either before or after implantation\textsuperscript{14}. Genital tract glycogen is unique in that unlike muscular glycogen, it is unaffected by either carbohydrate intake or exercises, but is controlled by ovarian steroids\textsuperscript{15}. Maeyama M et al found the glycogen content of the endometrium of normal and infertile patients was measured during the menstrual cycle. Glycogen content of the infertile group was significantly lower. Simultaneously urinary pregnanediol was measured which suggests that there is a high correlation between the function of corpus luteum and endometrial glycogen deposition\textsuperscript{16}. Basant Chaddha et al reported that glycogen levels were significantly decreased in the infertile women and repeated abortion. The decreased glycogen is significant as it is associated with lowered glucose content of the uterine fluid and cervical mucus, an important factor in sperm migration. As this may be corrected by hormonal therapy and thereby improve the fertility potential it may be worthwhile assessing this parameter in cases of unexplained infertility\textsuperscript{17}. 

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P M Sarren et al suggested that glycopenia in primary sterility patients is not primary but secondary to histological changes in the endometrium viz. greater percentage of proliferative and early secretory endometrial tissues\(^{18}\). B D Sabharwal et al observed the disparity in the distribution of glycogen in glands and stroma was also observed\(^{19}\).

The various factors responsible for low glycogen deposition have been suggested. They are low level of glycogen splitting enzyme, defective oxidation and utilization of glycogen may lead to environment, detrimental to the blastocyst. The amount of glycogen becomes more in secretory phase as there is shift from predominantly anaerobic glycolysis during proliferative phase to predominantly aerobic glycolysis during secretory phase. Decreased level of oestrogen and progesterone are responsible for the decreased deposition of glycogen in the endometrium, decreased permeability of cell membrane and no leakage of glycogen or glucose in the uterine fluid\(^{20,21}\).

Histologically the endometrium showed mild glycogen in 20\%, moderate 35\%, heavy 20\% & intense 25\% in infertility group in secretory phase as compared to control it was mild in nil, moderate in 5\%, heavy 17\% & intense 78\% cases. Also shows that the glycogen deficiency was seen mainly in luteal phase defect. This study suggests that deficiency of glycogen in the endometrium plays an important role in infertility which can be treated successfully with progesterone or clomiphine.

**Conclusion**

Our study shows the glycogen deficiency mainly in luteal phase defect, suggesting that deficiency of glycogen in the endometrium plays an important role in infertility.

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