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PRP and Egg Freezing Therapy for Early Puberty women

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Abstract

Egg freezing can be used for multiple reasons, however in this research we demonstrated the value of using it for women who suffer from early puberty. Besides that, PRP has been approved for broad clinical indications, and in our research, we investigated the possible use of it for women. whom suffer from LOR.

Materials and Methods

A clinical study was conducted in the British Syrian Medical Center for Assisted Reproduction and Fetal Medicine with a number of 137 women who suffered from early puberty, and we did not only take into consideration the age, but also many related factors such as hormones to be very accurate in our conclusion. And the parameters that were used to determine early puberty were:
1- Physiological body changes such as thelarche (breast development) and Pubic Hair.
2- The onset of menstruation.

Results

Out of 223 patients who had LOR, 137 patients (61.43%) had Early Puberty and 86 patients (38.57%) didn't have Early Puberty. Based on the comparison between AMH values for Normal Puberty and Early Puberty patients, we can build our conclusion that there is no correlation with ovulation reserve and the status of Early Puberty.

Conclusion

The menopause is not correlated to the status of early puberty and doesn't reflect a limited period of fertility. PRP can be definitely used to treat LOR in general for the first time worldwide, mainly in early puberty women and should be the first line of therapy.

1. Introduction

Early puberty is a significant medical as well as a social problem, however it should be understood under the light of normal pubertal timing is essential for any discussion of precocious puberty, yet remains a topic of intense debate. Historical analyses demonstrate that the age of menarche – a reliable reflection of HPG axis activation – dropped substantially in industrialized nations throughout the late 19th century and first half of the 20th century [1]. Most likely owing to improvements in overall nutrition and public health.

Studies up until the 1990s reported the onset of breast development, generally the first sign of true puberty, at about 11 years old, and the conventional definition of precocious puberty was onset prior to 8 years old in girls. However, the Pediatric Research in Office Settings study in 1997 reported that puberty (breast or pubic hair) was evident in 6.7% of White girls and 27.2% of African – American girls less than 8 years of age [2].

This study, along with National Health and Nutrition Examination Survey data and other surveys has redefined the mean age for onset of breast development as 10.0 years or less [3,4]. However, age at menarche remained unchanged except among African–American girls.

This research prompted revision of the Pediatric Endocrine Society

clinical guidelines to reflect a normal age at onset of puberty down to 6 years among African–American girls and 7 years among White girls [5]. However, the endocrine community has not fully embraced these guidelines due to two-fold concerns. First, the Pediatric Research in Office Settings study relied on visual inspection alone, and may therefore have detected adipose tissue from widespread obesity, rather than true breast development [6].

Second, many endocrinologists felt that lowering the age limit for normal 'puberty to this extent would result in an unacceptable rate of missed abnormality, a contention that has been supported by later studies [7].

More recent observations of only European cohorts have also reported a drop in the age of thelarche since the 1990s but could not support this by a significant change in age-based gonadotropin levels [8].

These results raise the possibility that secondary sexual characteristics may be occurring earlier in some children – whether due to obesity or environmental triggers of estrogenization-without activation of the HPG axis [9].

We also have reviewed some data which distinguished in between central and peripheral one.

Differentiating Central and Peripheral Precocious

Definition of Poor Ovarian Reserve

In the definition of POR by the BC, at least two of the following features must be present: advanced maternal age (□40 years), a previous poor ovarian reserve with □3 oocytes retrieved after conventional stimulation and/or an abnormal ovarian reserve test (ORT) [i.e. antral follicle count (AFC) < 7 or anti-Müllerian hormone (AMH) < 1.1 ng/ml]. In the absence of advanced maternal age or abnormal ORT, a patient can be defined as POR after two episodes of poor ovarian reserve following maximal stimulation.10 Initial studies found consistently low fresh live birth rates (LBR) among BC PORs. In particular, La Marca et al.11 included 210 PORs in a retrospective analysis and showed LBR ranging from 5.5% to 7.4%, while Polyzos et al.12 and Busnelli et al.13 also reported low LBR of around 6% [10-12].

In an effort to establish a common consensus for the definition of POR, in 2011 the European Society of Human Reproduction and Embryology (ESHRE) in the annual meeting proposed the Bologna'criteria [13]. According to these criteria, POR women should fulfill at least two out of the following criteria: a) advanced maternal age (above or equal to 40 years) or the presentation of other risk factors for an insufficient reserve, such as previous ovarian surgery, genetic defects, chemotherapy or autoimmune disorder, b) a former episode of poor ovarian reserve (oocyte yield of ≤3) following standard COS, and c) a low ovarian reserve with an antral follicle count (AFC) less than [5-7].

follicles and serum anti-mullerian hormone (AMH) less than 0.5–1.1 ng/mL. When other criteria are not present, recording at least two incidents of poor ovarian reserve following COS may serve as adequate justification to define a poor responder patient [14]. The abovementioned criteria constituted an original effort subject to precision systematic analysis in aptly defining patients who respond poorly to COS. interaction between poor responder and early puberty.

Within the statistics carried out by the British -Syrian İVF & FM Centre for 224 cases of patients with poor ovarian reserve, we found that of them, 137 cases of early puberty, which is equivalent to 61% [15].

5.4 Pregnancy comparison between a woman with precocious puberty and a woman without precocious puberty. Within the statistics carried out by the British -Syrian İVF & FM Centre for 224 cases of pregnancy women, we noticed that there were 8 cases of women who had a positive pregnancy result with early puberty, while we noticed that there were 14 women who had a positive pregnancy result without early puberty. From the above, early puberty has a clear impact on pregnancy [16].

The Effectiveness of Autologous Platelet-Rich Plasma PRP In the Therapy of Infertile Women with Poor Ovarian Reserve POR, A Retrospective Chart-Review Study.

Platelet-rich plasma (PRP), also known as autologous platelet gel, is "autologous blood with concentrations of platelets above baseline levels, which contains at least seven growth factors". plasma rich in growth factors (PRGF), and platelet concentrate (PC), is a high concentration of autologous platelets suspended in a small volume of plasma after centrifugation [17]. More than 800 types of protein molecules, cytokines, hormones, and chemo-attractants are carried by the platelets [18]. When platelets get activated, numerous biologically active proteins that stimulate cell proliferation, growth, and differentiation are released. Activated platelets also release various types of growth factors like platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β), fibroblast growth factor (FGF), epidermal growth factor (EGF), insulin-like growth factor (IGF-1), and hepatocyte growth factor (HGF) [19].

Cell ratios in normal blood contain only 6% platelets, however, in PRP there is a concentration of 94% platelets. With its rich growth factor composition, has been already proven beneficial in regenerative therapy [20]. Some of the specific beneficial effects of PRP are accelerated angiogenesis and anabolism, inflammation control, cell migration, differentiation, and proliferation were identified by a few previous studies [21,22]. As a result, PRP is nowadays used in various clinical scenarios that required improved tissue regeneration [23-25]. In maxillofacial surgery, neurobiology, orthopedics, sports medicine, and ophthalmology [19,26]. Potential benefits of PRP have also been studied in the field of gynecology; a study revealed PRP can increase endometrial thickness and improve the pregnancy outcome with

a thin endometrium [26]. According to Colombo GVL, PRP has the potential to reduce implantation failures by increasing the expression of adhesion molecules, and ovarian rejuvenation and folliculogenesis reactivation in peri-menopausal women [27].

To enhance the chance of pregnancy to whom having limiting factors for the success of any treatment modality for infertility as in "Poor ovarian reserve (POR)" which associated with a reduction in quantity of oocytes in women of reproductive age group. It may be age-related as seen in advanced years of reproductive life or may occur in young women due to diverse etiological factors.

Since the regulation of local health authority documented egg donation as a forbidden approach, Thus, a good number of women with POR need to undergo in vitro fertilization to achieve pregnancy. When the poor ovarian reserve overlapped with the problem of infertility in women, the interest in using assistive technologies to modify the results of ART in women with POR increased, and one of these therapeutic methods was rejuvenation Ovarian PRP, as sensational novel therapy has the potential to put a full stop to our long search for the question of poor ovarian reserve and getting a genetically linked baby. We review PRP to evaluate the effectiveness in the therapy of infertile women with poor ovarian reserve.

Finally, we still think that freezing egg will remail the ideal solution if financially possible mainly that PRP is also a new therapy which we hope it will be a guide line in LOR in America western world but like any new therapy it will take a while therefore freezing remain as a practice in advance but should remain even after such a promising therapy.

Egg freezing is one way of preserving a woman's fertility so she can try to have a family in the future. It involves collecting a woman's eggs, freezing them and then thawing them later on so they can be used in fertility treatment.

We propose this solution for all women who has early puberty and to used PRP 2 months before freezing (eggs and embryos) which is a first step for IVF in the proportion of women who shows poor Ovarian responder and we stress that to be a general guideline for all such cases independent of early puberty since the international medical community did not yet approve that despite the promising results in our medical center [28].

Doctors will need to tested for any infectious diseases like HIV and hepatitis for the women. but is to ensure that affected egg samples are stored separately to prevent contamination of other samples. You'll then start the IVF process, which usually takes around two to three weeks to complete. Normally this will involve taking drugs to boost your egg production and help the eggs mature. When they're ready, they'll be collected whilst you're under general an aesthetic or sedation. At this point, instead of mixing the eggs with sperm (as in conventional IVF) a cryoprotectant (freezing solution) will be added to protect the eggs. The eggs will then be frozen either by cooling them slowly or by vitrification (fast freezing) and stored in tanks of liquid nitrogen. Latest statistics show that vitrification is more successful than the slow cooling method. Most women will have around 15 eggs collected although this isn't always possible for women

with low ovarian reserves (low numbers of eggs). When you want to use them, the eggs will be thawed and those that have survived intact will be injected with your partner's or donor's sperm. [28].

Despite that we see this a possible option since we cannot predict the number of eggs for each woman we should mention here even the side effects of such process which is simple and not related to the women.

Risks That Woman May Face When Freezing

There are no risks that a woman may face except for some side effects that may be caused by drugs that stimulate and suppress the cycle.

IVF is mostly very safe, although some women do experience side effects from their fertility drugs. These are usually mild, but in extreme cases women who has ovulation reserve can develop ovarian hyperstimulation syndrome (OHSS), which is potentially fatal, so you should familiarize yourself with the symptoms.

The major risk is that it won't work – read more about success rates below.
It's also important to know that as you get older, there is more risk of pregnancy-related complications and health problems to both you and your baby [28].

Finally, How Successful is Egg Freezing?

Egg freezing is a rapidly changing field. If you do decide to freeze your eggs, make sure you choose a clinic that has plenty of experience and ask to see their most recent success rates for women your age.

When looking at success rates for frozen eggs, numbers tend to be quite low. The technology for egg freezing has also improved over the years which means older data isn't comparable to current success rates. We advise patients to look at success rates for fresh IVF cycles with patients using their own eggs in their age band. We consider these rates to be more reliable as there are much higher numbers of fresh embryo transfers each year compared to egg freezing. This information can be found in Choose a Fertility Clinic and in our Fertility trends report [28].

The standard storage period for eggs is for a maximum of 10 years, although women in certain circumstances may be able to store their eggs for up to 55 years for example, when the woman is at risk of becoming prematurely infertile through medical treatments such as chemotherapy.

Your clinician will be able to advise whether you meet the criteria for extended storage and explain what you need to do if you do meet the criteria. It is important to understand that currently if you are storing eggs for social reasons they can only remain in storage for up to a maximum of 10 years.

You must let the clinic know if you change address. This is important as if the clinic can't reach you at the end of the consent period, they may have to take your eggs out of storage and allow them to perish.

If you have the option to store for 55 years, you'll need to confirm that you want to continue storing your eggs and your doctor will need to confirm that you're eligible to do so. Again, it's vital that you stay in touch with your clinic to prevent your eggs from being discarded if your storage runs out [28].

Eggs that have been frozen and thawed must be fertilized using a fertility treatment called ICSI, as the freezing process makes the outer coating around the eggs tougher and sperm may be unable to penetrate it naturally under IVF.

This will be an extra cost on top of the fee for collecting, freezing and storing your eggs unless you have NHS funding [28].

Monitoring Ovarian Stimulation

Transvaginal ultrasound scanning:
No. & size of follicles
Pattern & thickness of endometrium
Estrogen blood level [31].

Ovulation Induction and Problems, We May Encounter

Complication of ovulation stimulation and IVF techniques
Complications can occur during ovulation induction, ovulation, or even after surgery, and ovarian hyperstimulation syndrome (OHSS) is one of the most serious complications that can face ovulation induction. The risk ranges from a simple condition to severe and may be life-threatening so that treatment is required in the hospital, the patient may develop this syndrome a few days after receiving the hormone human chorionic gonadotropin (hCG), and this condition is called (early hyperexcitable syndrome), and it may occur in It is called (delayed hyperexcitability syndrome) [30].

Multiple pregnancies are associated with an increased risk of delayed syndrome.
Other complications associated with IVF other than ovarian hyperstimulation syndrome (OHSS) have not been reported, except in isolated, exceptional cases [30].

The Required Analyzes to Be Carried Out Before the Ovarian Stimulation Process

AMH: anti molarian hormone
CBC : Complete Blood Count with Differential
FSH:Follicle-stimulating Hormone
TSH:Thyroid-stimulating Hormone
PROLACTIN
TOTAL AND FREE T4:L-THYROXINE SERUM CONCENTRATION
Estradiol And Other Forms Of Estrogen

Induction Protocols

There are two most commonly used GnRH agonist protocols. The first one is long agonist downregulation GnRH agonist protocol (Fig. 5.1). In this protocol, GnRH agonist is given in the mid-luteal phase of previous cycle. The second one is the short fl are agonist downregulation protocol, where the GnRH agonist is given on day 2 of menstruation (Figure 5.2) [32]. In the long protocol, a GnRH agonist depot preparation or daily injections are initiated during the mid-luteal phase of the preceding cycle. Today, this protocol, which aims at complete desensitization of the pituitary gland before starting stimulatory therapy, is still the most used worldwide for assisted reproductive techniques. Despite its effectiveness in preventing the premature LH surge and allowing strict cycle control, it has some disadvantages such as the following

- Duration: treatment cycle at least 14 days longer than the normal menstrual cycle.
- Administration of the agonist in the presence of a possible early pregnancy.
- Cyst formation: the fl are-up effect might interfere with ovarian function.
- Hormonal withdrawal symptoms.
- Gonadotropin use: more gonadotropins are used as compared with cycles without the use of GnRH agonists.
- Ovulation induction only possible by human chorionic gonadotropin (hCG) or LH, but not by GnRH due to desensitization of the pituitary gland.
- Need of luteal phase support due to desensitization of the pituitary gland.
- Increased incidence of moderate and severe OHSS as compared with cycles without the use of GnRH agonists.
- Disturbance to subsequent menstrual cycles due to prolonged pituitary suppression after desensitization [33].

The GnRH agonist protocol may result in stable and low LH and progesterone (P) levels throughout the stimulation phase and may also cause suppression of endogenous FSH levels, leading to a follicular cohort of all small follicles at the initiation of FSH stimulation resulting in a synchronized follicular development. The advantages of this protocol are increased number of oocytes collected, additional pregnancy chances from cryopreserved embryos, and improvement in patient scheduling [34].

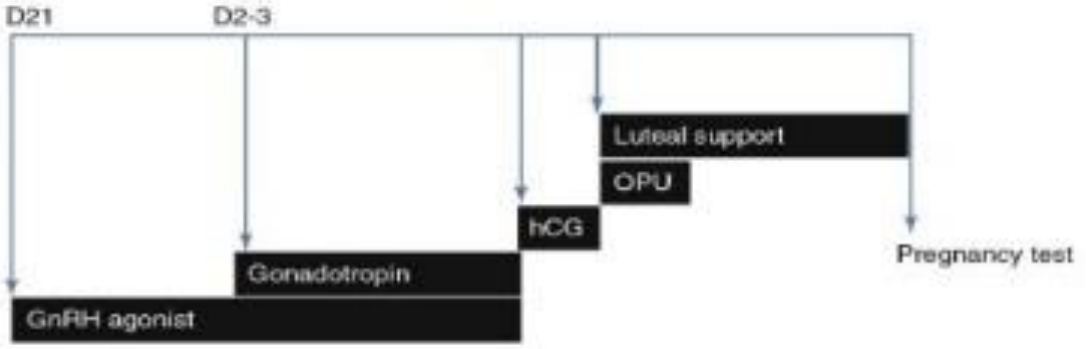

Figure 1: GnRH Agonist Downregulation Protocol

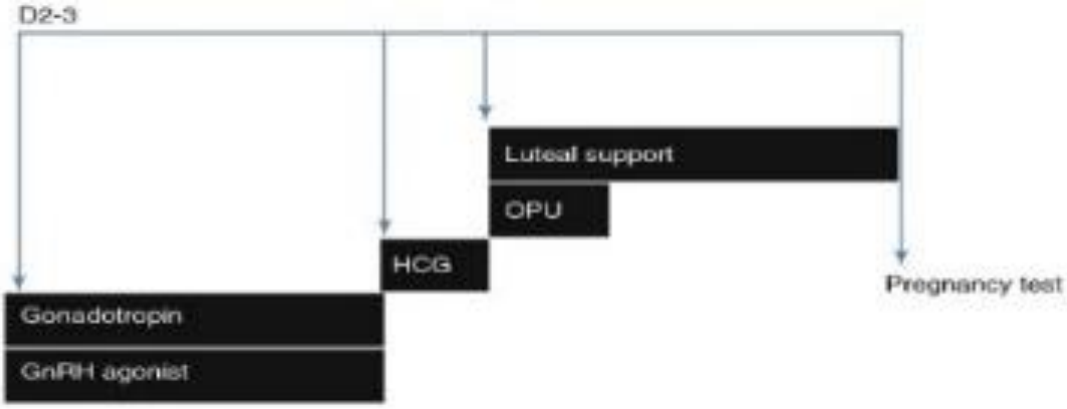

Figure 2: Short Flare GNRH Agonist Protocol

Oocyte Recovery

Today, the process of withdrawing eggs is performed under general anesthesia or by venous analgesia, and with the help of ultrasound imaging. The patient lyes on her back with knee flexion taking into account sterilization and coverage. Then a vaginal probe attached to the needle guide is inserted into the vaginal to photograph the ovaries and follicles meanwhile, the drag device is prepared, ensuring the compressive strength (100 mmHg) by discharging the nutrient medium as a test of the permeability of the specially manufactured needle for single use for this purpose. The work begins with the closest ovaries, so the needle is inserted into the first follicle with the help of photocontrol. The follicular fluid is drawn into a test tube that is placed inside a warm device, and then delivers the test tube to the embryologist who is waiting in the IVF laboratory attached to the operating room. All follicles at one end should be discharged regularly without washing, very careful all the time not to compromise the vessels near the pelvic wall. After completing the discharge of all the follicles at one end, the needle is pulled and used to pull a little of the feeder medium into a special test tube to wash inside the needle. The surgeon then moves to the opposite ovary and enters the needle again, proving with the image of waves, to empty the remaining follicles and also the follicles are completely emptied and discharged. After finishing, pull the needle and wash the device back with the warm implant medium to ensure that the eggs do not remain inside the needle device. The uterus is scanned with the thickness of the lining measured before removing the imaging probe. Wash the vagina with a piece of sterile gauze and make sure the bleeding stops. After the operation, the patient is placed under observation in the resuscitation room, and re-examined after 2-6 hours, before being discharged from the hospital.

When the embryologist receives the test tube containing follicular fluid, its content is poured into a Petri Dish, and examines its components under anatomical Binocular Dissecting Microscope to investigate the presence of the OCC. After those compounds have been determined, the substance is immediately transferred to a Buffered Medium protective nourishing medium, where it is washed three times to remove red blood cells before being transferred to the nourishing medium dish containing an artificial medium monitored for living tissue transplantation. Then the dish containing the egg rubble is placed inside an incubator until the time of vaccination, 4 hours on the egg. After the passage, the eggs are classified according to the expansion of the egg rubble and the severity of the cohesion of the coronal cells [29].

Egg Freezing

Eggs are frozen after the withdrawal process followed by Denudation erosion.

Where the maturation of eggs is monitored and the eggs are frozen only within a mechanism adopted by each laboratory according to the materials used for freezing.

the most prominent stages are:
1. Maturity control
2. Placing eggs inside straw
The straw accommodates a limited number of eggs.
1. Eggs are transported to liquid nitrogen - 1969 (Oral communication with Dr Rami Obeid Alnasser)
Note: Eggs are frozen on the same day of withdrawal.

Materials and Methods

In the British Syrian ivf center to help fertility. A clinical study was conducted with a number of women who suffered from early puberty , and we did not only take into consideration the age, but also many related factors and hormones to be very accurate in our conclusion.

Results

Figure (3) shows that out of 223 patients who had LOR, 137 patients (61.43%) had Early Puberty and 86 patients (38.57%) didn't have Early Puberty.

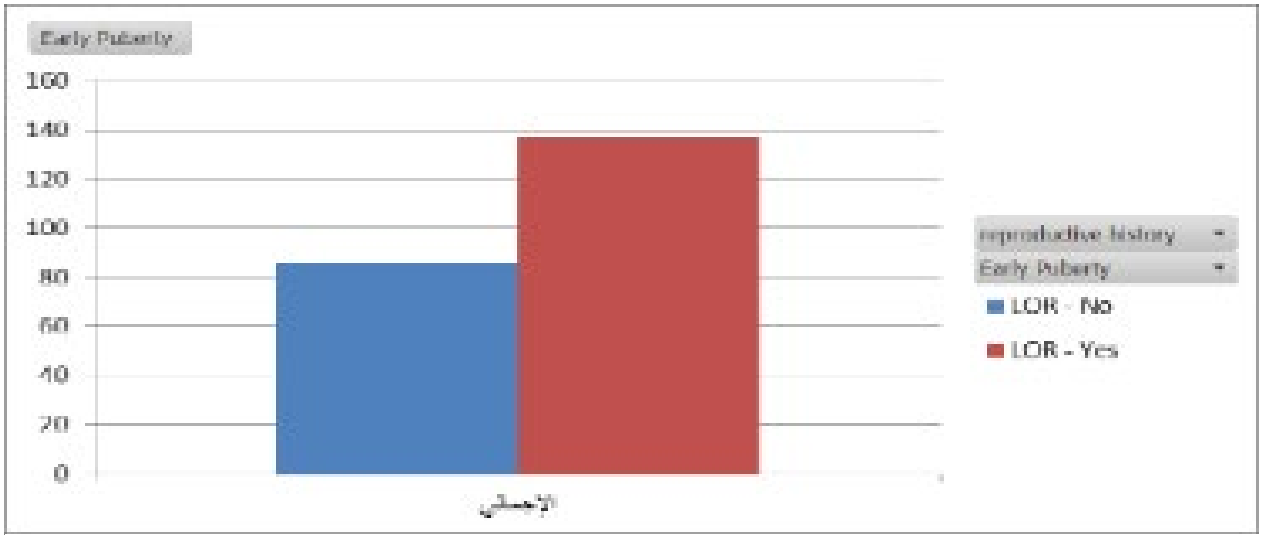

Figure 3: Relationship between LOR and Early Puberty

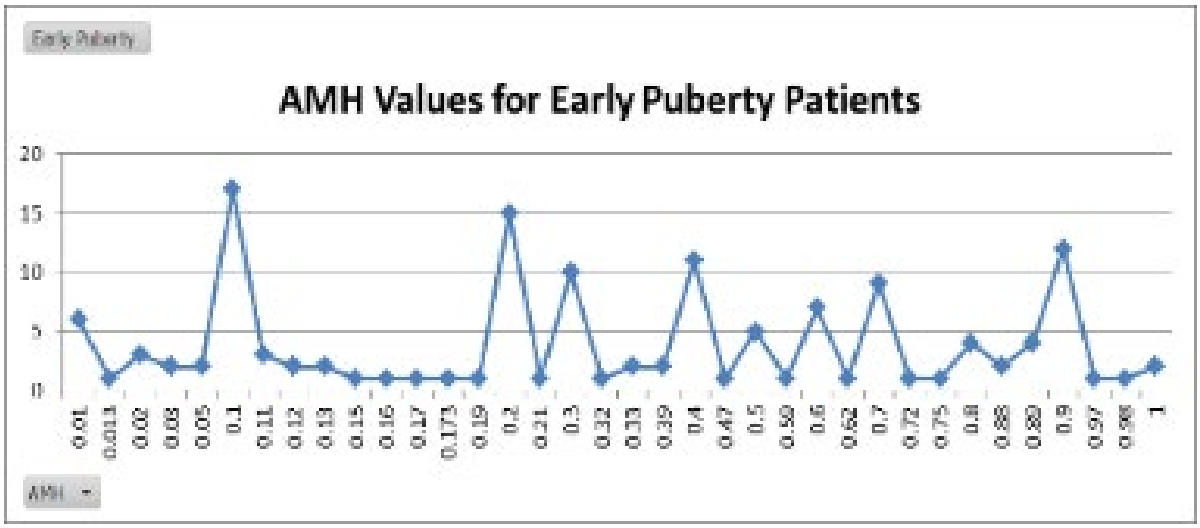

Figure 4: AMH values for all Early Puberty Patients

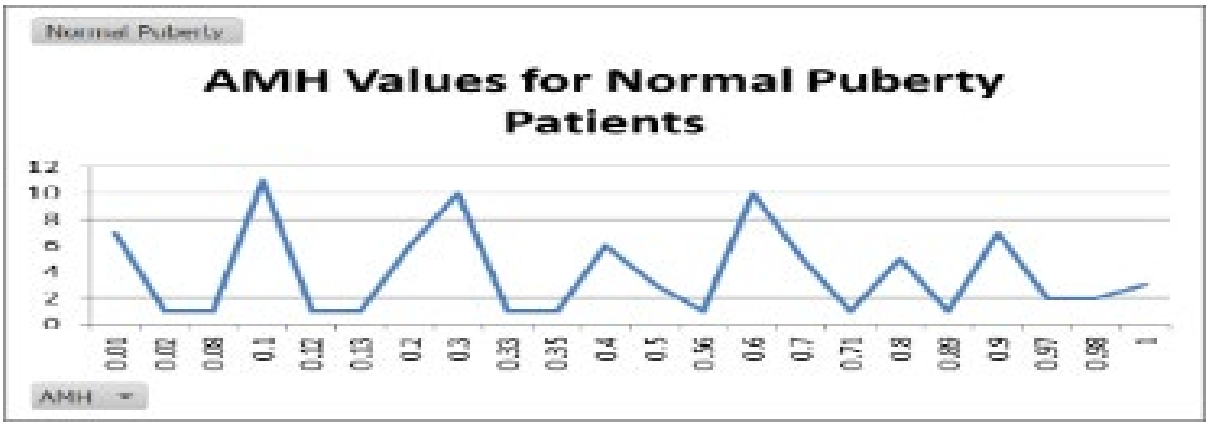

Figure 5: AMH Values for Normal Puberty Patients

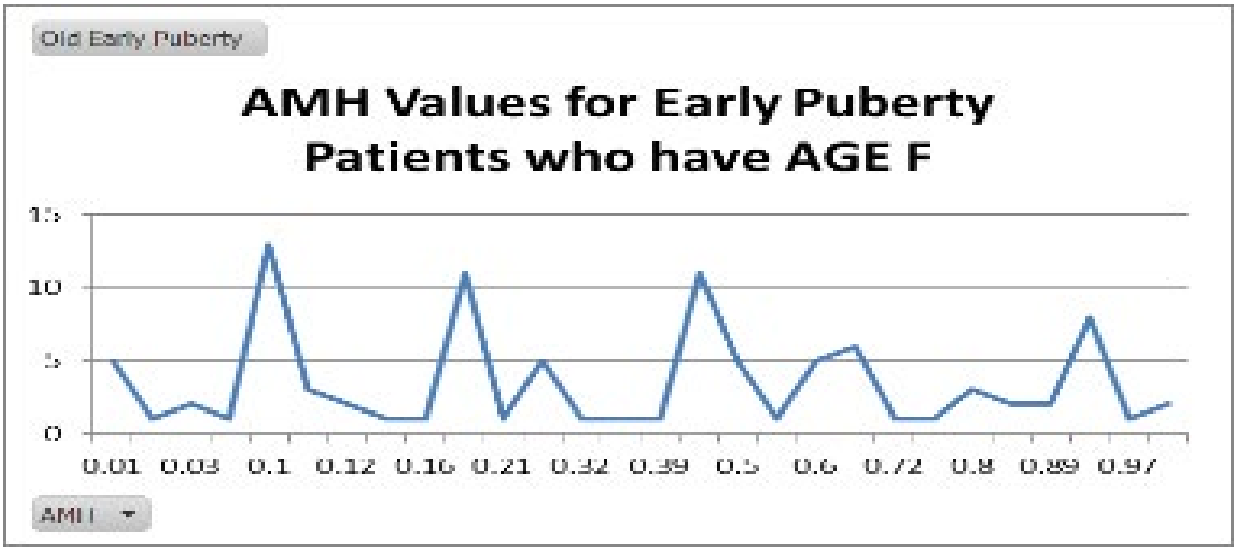

Figure 6: AMH Values for Early Puberty Patients who have AGE F >35 years old

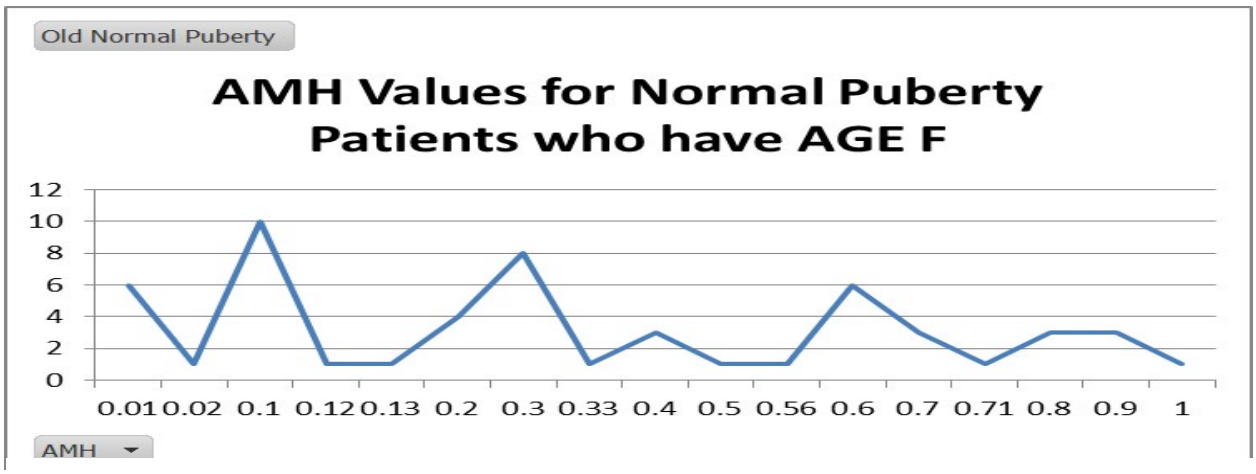

Figure 7: AMH Values for Normal Puberty Patients who have AGE F >35 years old

Conclusion

The goal of our research was to investigate the rate of early puberty patients, and based on the comparison between AMH values for Normal Puberty and Early Puberty patients, we can build our conclusion that there is no correlation between the ovulation reserve (AMH) and the status of Early Puberty.

Since the major concern in early puberty normally could cause shorter fertility therefore women are forced to be involved in a rapid and likely unproper marriage which was not observed according to our clinical study which demonstrated that the ovulation reserve is variable in early puberty which is an important conclusion therefore we should increase public awareness about that women. However, egg freezing is possible it remains the ideal solution after fertility tests (AMH) since the count of the number of eggs is not available in clinics for women in general.

Therefore, the goal of our study is to highlight this issue and its possible solution however by reviewing figure (3) we noticed that women who suffered from early puberty have (61.43%) of those women who have poor ovarian responder which could be treated by PRP in order to induce ovules before freezing them.

The use of PRP has been approved for clinical use but this is the first time ever it succeeded in treating such an important disease (LOR), and it was very effective. [17-28]. And we stress that it should be the first line of therapy in such diseases worldwide.

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