

Triplet Birth **after** Only GnRH Depot Agonist Down Regulation Using **TESE** Sperms In **An** IVF-ICSI **Case**

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A successful triplet birth of in vitro fertilization following only down-regulation using gonadotrophin –releasing hormone analogue (Goserelin depot) administration in the early follicular phase of the menstrual cycle is reported. The patient underwent an intracytoplasmic sperm injection using fresh testicular biopsy due to non-obstructive azoospermia of the husband. No gonadotrophins injections were used to stimulate the ovaries. The events in such a case are described with review of the literature.



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Objective: Here is a report of a live triplet birth after down-regulation with GnRH agonist alone, resulting in multifollicular formation and using fresh TESE Sperms from a husband with non-obstructive Azoospermia.

Setting: Private –based infertility center.

Patient(s): A 30-year-old Yemeni woman (gravida 1, para0) and a husband with non-obstructive azoospermia.

Interventions: Only down-regulation using gonadotrophin–releasing hormone analogue (Goserelin depot) administration in the early follicular phase of the menstrual cycle is reported. An intracytoplasmic sperm injection using fresh testicular biopsy due to non-obstructive azoospermia of the husband 36 hours after an hCG injection.

Main outcome measures: Number of follicles developed, number of and maturity of oocytes retrieved, development of clinical pregnancy, healthy triplet newborn.

Results: 10 days after administration of gonadotrophin – releasing hormone analogue (Goserelin depot) in the early follicular phase of the menstrual cycle for down-regulation to prepare the patient for an ovulation induction in an IVF-ICSI cycle. 7 metaphase II oocytes were retrieved and injected. All seven were fertilized using fresh motile testicular sperms. 48 hours later, 3 Embryos at 4-cell stage type I were transferred. 10 days after embryo transfer, Beta HCG was 83 mIU/mL and 2 days after that the Beta HCG went up to 221.52 mIU/mL. On January 18th, 2001 -40 days after embryo transfer- 3 gestational sacs were seen with evidence of 3 fetal heartbeats. On August 9th, 2001 and at exactly 35 weeks after embryo transfer the triplets were born by caesarian section.

Conclusions: down-regulation with GnRH-a at D1 of the menstrual cycle had developed multiple follicles (7 mature and equal sized follicles in this

reported case). Using testicular sperms in this case of non-obstructive Azoospermia lead to fertilization of all the collected oocytes. The end result was triplet birth (two boys and one girl).

Introduction

Gonadotrophin-releasing hormone (GnRH) is a hypothalamic decapeptide that plays a central role in female repro-

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ductive functions. Its secretion is in a pulsatile manner from the hypothalamic neurons. It gets bind to specific receptors on pituitary gonadotrophs, thus leading to intermittent secretions of both (FSH) follicles stimulating hormone and (LH) luteinizing hormone. The use of gonadotrophin-releasing hormone agonist (GnRHa) in assisted reproductive technologies has been generally accepted as the best way to induce multiple follicular development in combination with exogenous gonadotrophins. In female reproductive organs, the actions of GnRH have been shown to be predominantly inhibitory. These include inhibition of ovarian steroidogenesis, ovulation, ovum transport, ovum implantation, pregnancy and uterine growth (Hsueh, and others, 1981).

Clinical application of GnRH and its analogues falls into 2 broad categories: that dependent upon inhibitory effects on gonadotrophin secretion and those dependants upon stimulatory effects of GnRH on gonadotrophin secretion (Gordon and others, 1993). Gonadotrophin-releasing hormone agonists (GnRHa) are now used in con-

junction with exogenous gonadotrophin as an integral part of most ovulation induction protocols for various forms of assisted reproductive technologies (ART).

Case Report

A 30-year-old Yemeni female presented to our center with her husband as a case of primary male infertility of 18 years duration. She had menarche at the age of 12. Menstrual cycle was regular, Duration/ period 30/5 days, with occasional dysmenorrhoea. No previous history of any illnesses or surgical procedures. No family history of any illnesses. Transvaginal ultrasonography revealed an anteverted uterus of normal size and free of masses, right ovary of 40.1mm x 16.1 mm and left ovary of 20.9mm x 27.0 mm. There were more than 7 small sized follicles scattered on each ovary on D3 of the cycle, but not PCO appearance was noticed. D3 hormone profile was FSH 4.4 mIU/ml, LH 2.5 mIU/ml, and E2 46 pg/ml.

Seminal fluid analysis of the husband showed azoospermia. Hormone profile for the husband was FSH 39.2mIU/mL, LH 28.2 mIU/mL Testosterone 0.6 ng/mL (low) using Axsym analyzer (Abbott Diagnostic). 6 testicular biopsies from both testes were taken from the husband before starting any in vitro fertilization protocol to confirm the presence of spermatozoa in the testis.

The patient received a long acting GnRH-a depot injection (Zoladex "Goserelin Depot" 3.6 mg, Astra-Zenica, England) on D1 of the menstrual cycles as an early follicular phase down-regulation. The patient came back 10 days later for further ovulation induction. Transvaginal ultrasonography using Medison SONOACE 6500, Color Doppler ultrasonography, with a 6.5 MHz vaginal probe revealed an endometrial thickness of 13.2 mm and that the right ovary contained 4 follicles of 21.5mm, 20.6 mm, 20.4 mm, and 18.9 mm. In the left ovary 3 follicles were seen, 21.9mm, 20.8mm and 19.2 mm. E 2 level was 2169 pg/ml. LH was

8.9mIU/ml and P4 was 0.79. Patient was given human chorionic gonadotrophin (HCG, Profasi; Serono) (10000 IU i,m) and 36 hours later 7 oocytes all at MII phase were retrieved. The husband underwent TESE, and ICSI was performed using fresh motile sperms.

48 hours later 7 Embryos were seen, 2 at 2 cell stage type I, and 5 at 4 cell stage type I. 3 embryos at 4 cell stage type I were transferred back to the uterus using Frydmann E/T Catheter under ultrasound guidance. Luteal phase support started one day after oocytes retrieval with Cyclogest suppositories 400 mg once daily, Duphaston 2 tablets twice daily and Baby Aspirin (100mg) once daily.

On December 19th, 2000 -10 days after embryo transfer, Beta HCG was 83 mIU/mL and 2 days after that the Beta HCG went up to 221.52 mIU/mL. On January 18th, 2001, 3 gestational sacs were seen with evidence of 3 fetal heartbeats

There were no complications during the pregnancy and regular Antenatal care was performed. A healthy triplet (Two boys each weighing 2200 gm and one

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girl weighing 2250 gm) were born on August 9th, 2001 (37th week), through Cesarean section.

Discussion

There has been no reports in the medical literature that GnRH analogues used alone to induce ovarian superovulation for in vitro fertilization cases and ending

in multiple bilateral ovarian follicle formation. It had always been used in conjunction with gonadotrophins in all controlled ovarian hyperstimulation induction protocols. Also there are no reports of pregnancies resulting from the transfer of oocytes collected after hyperstimulation with GnRH-a alone. (Khalaf Y and others, 2000).

This case represents a rare an unusual ovarian response using a depot GnRH-a alone for down-regulation at early follicular phase

This is the first case reported with this outcome. Using GnRH-a alone in down-regulation and ending up with mature multifollicular formation rarely happens. Also in our case the husband was having non-obstructive Azoospermia and the sperms were retrieved by testicular biopsy.

Fertilization after the microinjection of oocytes collected from patients with the use of GnRH-a alone was reported (Khalaf and others, 2000, Weissman A and Others, 1998).

(Khalaf and others, 2000) mentioned that embryos that develop under such circumstances are capable of withstanding freezing and thawing processes.

As suggested by (Khalaf and others, 2000) several possible mechanisms were proposed for the development of ovarian hyperstimulation after the use of GnRH-a alone. Subtherapeutic doses of these agonists as suggested by (Navot and others, 1991) can cause ovarian hyperstimulation. A direct effect of GnRH-a at the ovarian level is possible mechanism particularly as GnRH receptors have been demonstrated in ovarian tissue. Variations in the number of these receptors, their affinity, and their modulation by growth factors may account for the ovarian response (Khalaf and others, 2000).

The timing of GnRH-a administration during the menstrual cycle may influence the time course of ovarian suppression and IVF outcome (Ferrareti and others, 1996). Using long protocol with down-regulation at early follicular phase usually excludes pregnancy when GnRH-a administered and is easy to organize (Ravhon and others, 2000).

The agonist phase of treatment with GnRH-a can be troublesome with regards to stimulating the ovary to develop follicular cysts. The agonist phase is more prolonged when GnRH-a is started in the follicular phase. Suppression is more prompt and consistent when GnRH-a is started in the early follicular phase. (De Fazio and others, 1985).

In one investigation there was a trend towards improved embryo morphology when the long protocol was begun in the early follicular rather than mid-luteal phase, and the required of GnRH-a was lower. (Ron El R and others, 1990)

This case represents a rare an unusual ovarian response using a depot GnRH-a alone for down-regulation at early follicular phase. This is the first reported cases that ends up with multiple gestation and a testicular sperms used in a case of non-obstructive Azoospermia.

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