Comparative study between subcutaneous and intramuscular administration of human chorionic gonadotropin during induction of ovulation in ICSI as regards its serum and follicular fluid concentrations and the effect on the maturity of ova.

Thesis

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Introduction:

The achievement of a simple, safe and cost-effective treatment protocol in controlled ovarian hyperstimulation (COH) is of paramount importance to improve the quality of care in assisted reproduction. The midcycle gonadotropin surge is a major event in the dynamics of ovulation. Rapidly increasing levels of luteinizing hormone (LH) induce a number of key changes in both oocytes and follicular cells, which further modify the steroid and protein micro- and macroenvironment. These physiologic changes have a prominent role in the normal maturation of oocytes, the process of ovulation, and in subsequent fertilization and implantation (**Shoham Z el al.,1995.)**

Human chorionic gonadotropin (hCG) has been used as a substitute for the LH surge because of the degree of homology between the two hormones. **(Chandrasekher, et al., 1994).**

In assisted reproductive procedures, hCG is precisely timed and often given at night, which means the patient must have someone available outside of normal office hours to administer her IM injection. If hCG could be injected subcutaneously, this would greatly lessen the stress for the patient. [**Elkind-Hirsch et al., 2001**]

Now the highly purified gonadotropins are now used SC, requiring that patients learn how to correctly inject themselves by two techniques, IM and SC. This leads to patient confusion, discomfort, and increased need for patient education. Subcutaneous injection of purified hCG received very little attention to date. There are few studies that evaluate the pharmacodynamics of hCG injection. [**Stelling et al., 2003**]

It was found that the serum levels of hCG required for normal follicular maturation are likely to be achieved through the SC route. [**Saal et al., 1991**]

After reviewing 600 IVF cycles, it was reported that hCG may be given by SC administration with an efficacy similar to IM injection. [**Frunkfurter et al., 1998**]

As regards the effect of hCG level, Nagata et al., studied serum and follicular fluid levels of hCG administered IM. They concluded that the ratio of the level of hCG in the follicular fluid to the level of hCG found in the serum was a good marker for ovarian blood flow and therefore for ovarian responsiveness and subsequent pregnancy. [**Nagata et al., 1999**]

Also, Wikland et al. investigated the route of administration of hCG to women undergoing IVF; patients

were randomly allocated to receive Profasi SC or I.M. A variety of endpoints as the number of oocytes, fertilization rate, number of transferred embryos, and number of pregnancies were evaluated with the primary clinical measure being oocyte maturity. No differences were seen as regards number of mature oocyte and all IVF outcome data between IM and SC groups. [**Wikland et al., 1995**]

Gonadotropins:

Human chorionic gonadotropins (hCG):

The discovery of hCG:

In 1927 **Aschcim** and **Zondek** demonstrated that the blood and urine of pregnant women contained a gonad-stimulating substance; injecting this substance subcutaneously into intact immature female mice produced follicular maturation, luteinization and hemorrhage into the ovarian stroma. This became known as the **Ascheim-Zondek** pregnancy test. **Ascheim** and **Zondek** believed that this gonadotropic substance was produced by the anterior pituitary.

Subsequent work showed that this gonadotropin was produced in vitro in placental tissue culture, proving conclusively that the placenta and not the pituitary was responsible for the elaboration of the hormone. This gonad-stimulating property was exhibited by the chorionic villi, and was especially marked in the cytotrophoblastic Langerhans cells. (**Seegar-Jones et al., 1943).**

Marius Tausk's book on the history of Organon, **(Tausk M, 1978)** describes the gonadotropic hormone hCG (extracted from human placenta) as being very similar to the pituitary hormone 'Prolan B' = LH. Organon launched this extract on the market in 1931, under the name 'Pregnon'. However, because of similarity with another trademark, the name was later changed to 'Pregnyl'.

Pregnyl was released in 1932.

According to Tausk this preparation was used for 'stimulation of the ovaries' at first, and the initial hCG products were calibrated in animal units. A rat unit was defined as the smallest amount that produced vaginal opening together with estrus when injected into female immature rats.

The International Standard for hCG was established in 1939, under the auspices of the League of Nations. The international unit (IU) was defined arbitrarily as the activity contained in 0.1 mg of the standard preparation. **(Bruno Lunenfeld and Kay Elder, 2005)**

Purified urinary preparations of hCG became available in 1940 **(Guirin et al., 1940)**. The potency of these preparations ranged from 6000 to 8500 lU/mg. **(Katzman et al., 1943).**

Clinical studies with hCG began as early as 1933 **(Hamblen, 1933)**, and were summarized by the same author 15 years later **(Hamblen, and Ross 1947),** women who were scheduled for non-gynecological abdominal surgery were injected with hCG, and the ovaries were inspected during the operation. When hCG was administered in the follicular phase of the cycle, their ovaries showed no evidence of follicle stimulation, ovulation or corpus luteum formation, i.e. in the absence of FSH, no visual effect of hCG could be seen.

Biochemistry of hCG:

HCG is a member of the glycoprotein hormone (GPH) family, which also comprises LH, FSH and TSH. All GPHs are heterodimers consisting of an -subunit (GPH) and a ß-subunit. The -subunit, which contains 92 amino acids, is common to all GPHs. The ß-subunits confer biological activity and display various degrees of homology, which between hCG and LH is 80%. LHß contains 121 amino acids whereas hCGß contains 145 amino acids, the difference being due to a 24-amino-acid extension, the so-called C-terminal peptide (CTP) **(Pierce and Parsons, 1981).**

One-third of the mass of hCG is made up by eight carbohydrate moieties, of which six are attached to hCGß and two to hCG . The N-linked carbohydrate chains on hCG are attached to Asn52 and Asn78 and those on hCGß to Asn13 and Asn30. Four O-linked oligosaccharides are attached to Ser121, Ser127, Ser132 and Ser 138 on the CTP of ßhCG **(Kessler et al., 1979 a, b ; Elliott et al., 1997).**

Because of heterogeneity of the CHO moieties, the molecular weight (MW) displays a spectrum of values. The average MW of hCG determined by MALDI-TOF mass spectrometry is 37 500, that of hCG is 14 000 and that of hCGß 23 500. **(Birken et al., 2003).**

Metabolism of hCG:

The clearance of hCG from circulation has been

studied both after injection of purified hCG and after pregnancy. The half-life of injected hCG is biphasic; the rapid phase has a half-life of 5–6 h whereas that of the slower phase is 24–33 h. **(Rizkallah et al., 1969; Wehmann and Nisula, 1981)**.

Similar clearance rates have been observed after an abortion and term pregnancy, but the clearance is best described by a triphasic model with median half-lives of 3.6, 18 and 53 h **(Korhonen et al., 1997).**

The half-life of purified hCGß injected into humans is 0.7 and 10 h, which is shorter than that of hCG **(Wehmann and Nisula, 1979).** However, after term pregnancy or an abortion, hCGß actually disappears more slowly than hCG with half-lives of 1, 23 and 194 h.

The half-life of hCG is shorter than that of hCGß, and after term pregnancy half-lives of 0.6, 6 and 22 h have been observed **(Korhonen et al., 1997).** These half-lives are longer than those observed after injection of purified hCG , i.e. 0.1–0.22 and 1.2–1.3 h (**Wehmann and Nisula, 1979; Blithe and Nisula, 1987).**

The discrepancy between half-lives determined for injected and naturally occurring subunits may indicate that the purified forms have been partially denatured during purification, and they are therefore metabolized more rapidly. It is also possible that the slower metabolism of endogenous free subunits is explained by differences in

glycosylation.

Most of the hCG in circulation is metabolized by the liver, whereas about 20% is excreted by the kidneys **(Nisula et al., 1989).**

During excretion, a major part of hCG is degraded to subunits, nicked forms and especially hCGßcf **(Wehmann and Nisula, 1980; Nisula et al., 1989)**.

After injection of urinary hCG, hCGß or recombinant hCG (rhCG), hCGßcf is detected in urine **(Nisula et al., 1989),** but peak concentrations occur 6 h after the hCG peak in urine **(Norman et al., 2000).**

Therapeutic uses of hCG in induction of ovulation::

The midcycle LH surge is essential for normal oocyte maturation and ovulation. In ART, administration of partially purified urinary hCG preparations has been used for decades as a surrogate for LH to achieve final oocyte maturation and ovulation in controlled ovarian hyperstimulation (COH) protocols. This facilitates correct timing of oocyte retrieval in connection with IVF/ICSI treatments.

Urinary hCG has been the drug of choice, but there are now other options, i.e. recombinant LH (rLH) and hCG (rhCG). rLH has been available for use in clinical trials for several years. A single dose of 15 000 – 30 000 IU of rLH gives the highest efficacy to safety ratio. Such a dose is comparable with 5000 IU of urinary hCG, and it effectively

induces final follicular maturation and early luteinization in IVF embryo transfer patients **(The European recombinant LH study group, 2001).**

However, rLH has not been shown to be superior to hCG in clinical practice. Moreover, it has been suggested that replacement of urinary hCG by rLH in agonist cycles results in a significantly lower pregnancy rate **(Aboulghar and Al-Inany, 2005).**

There is no evidence of difference between rhCG or rhLH and uhCG in achieving final follicular maturation in IVF, with equivalent pregnancy rates and OHSS incidence. According to these findings uHCG is still the best choice for final oocyte maturation triggering in IVF and ICSI treatment cycles. **(Youssef et al., 2011)**

For many years, the most widely used form of COH in IVF/ICSI protocols has been to use GnRH agonists to desensitize the pituitary and suppress gonadotropin secretion, followed by ovarian stimulation with FSH and/or HMG. Presently, GnRH antagonists are an alternative in preventing premature LH surges in COH. Because the pituitary remains responsive to GnRH agonists, administration of a bolus of GnRH agonist induces an endogenous LH surge **(Griesinger et al., 2006).** This form of treatment has been suggested to prevent the ovarian hyperstimulation syndrome (OHSS) **(Orvieto, 2005).**

In clinical studies, ovulation induction with the

GnRH agonist, Buserelin, resulted in significantly more mature oocytes, but significantly lower implantation and clinical pregnancy rates were obtained than those by conventional ovulation induction with urinary hCG **(Humaidan et al., 2005).**

Moreover, the rate of early pregnancy loss was higher, probably due to luteal phase deficiency. This has been confirmed in other studies, and a lower probability of ongoing pregnancy was achieved with the GnRH agonist Triptorelin than with urinary hCG **(Kolibianakis et al., 2005)**. At present, hCG appears to be the most reliable way to trigger final oocyte maturation both in antagonist and in agonist cycles **(Griesinger et al., 2006).**

It seems that similar characteristics and dynamics of luteal phase Estradiol (E_2) and Progesterone are obtained after ovarian stimulation for IVF using GnRH agonists or antagonists **(Friedler et al., 2006),** and thus luteal support is needed in both protocols. Luteal phase support after ART results in an increased pregnancy rate compared with placebo or no treatment. When hCG is compared with progesterone treatment, there is no significant difference in pregnancy rates but hCG is associated with a greater risk of OHSS **(Daya and Gunby, 2004).**

Urinary hCG can also be used for other clinical applications. Thus low-dose hCG has been used alone to complete controlled ovarian stimulation **.** When used in the

late stage of ovarian stimulation (after the follicles were 12 mm), this reduces FSH/HMG consumption while fertilization outcome is comparable. Furthermore, hCG use is associated with a reduced number of small pre-ovulatory follicles, which could reduce the risk of OHSS **(Filicori et al., 2005).**

Substitution of rFSH by low-dose **hCG** in the final days of COS leads to a reduction of FSH consumption whereas ICSI outcome, in terms of oocyte yield and ongoing pregnancy rate, remains comparable to the traditional regimen. **(Blokeel et al., 2009).**

Even more interesting is the effect of hCG on uterine receptivity. In a study, administration of hCG to oocyte recipients was shown to increase endometrial thickness on the day of embryo transfer and to improve the implantation rates **(Tesarik et al., 2003).** This suggests that hCG might affect endometrial function independently of ovarian function by stimulating endometrial growth and maturation and by enhancing endometrial angiogenesis, thereby extending the implantation window **(Filicori et al., 2005).**

Endogenous hCG plays a significant role in maintaining Leptin production in human placental syncytiotrophoblasts, and this effect involves a cross talk between cAMP and p38 MAPK pathways. And Leptin produced by the placental syncytiotrophoblasts participates in a number of processes in pregnancy including

implantation, proliferation of the cytotrophoblasts, and nutrient transfer across the placenta**. (Ge Y C et al., 2011**). And this explains the effect of hCG on implantation and pregnancy.

Although the incidence of failed hCG injection is rare, it was found that cycles characterized by incorrect initial administration or failed absorption of hCG can be salvaged by early detection and repeat injection. Assisted reproductive technology (ART) programs may benefit their patients through the assessment of either urine pregnancy tests or measurement of quantitative serum β-hCG levels before retrieval, thereby preventing empty follicle syndrome. **(Reichman et al., 2012)**

Another therapeutic use of hCG is triggering ovulation in ovulatory patients with natural cycles (NC) undergoing frozen-thawed embryo transfer (FET). It was found that triggering ovulation by hCG can significantly reduce the number of visits necessary for scheduling embryo transfer without an adverse effect on cycle outcome. Ovulation triggering can increase both patient convenience and cycle cost effectiveness, **(Weissman et al., 2011)**

Recombinant hCG (rhCG) :

hCG produced by recombinant techniques in Chinese hamster ovary cells has become commercially available. In ART, a dose of 250 µg of rhCG has been found to be

equivalent **(Chang et al., 2001)** or at least as effective as 10 000 IU of urinary hCG in inducing final stages of oocyte maturation. Furthermore, the use of rhCG was associated with significantly better patient tolerance **(Abdelmassih et al., 2005).**

The content of hCG in urinary preparations for therapeutic use is expressed in IU based on bioactivity whereas that of rhCG is expressed in mass units **(Gervais et al., 2003)**, which complicates comparison of dosage. In animal and clinical studies, 250 µg of rhCG has been found to have the same biological activity as 5000 IU of urinary hCG **(Gervais et al., 2003; Al-Inany et al., 2005).** This would translate into a specific activity of 20 000 IU/mg, which is higher than the potency of 15 000 IU/mg of the most pure urinary hCG preparations presently available **(Birken et al., 2003).**

However, the content of rhCG is based on the mass of the peptide moiety only **(Gervais et al., 2003),** and thus 250 µg corresponds to 360 µg of glycosylated hCG and a specific activity of 13 900 IU/mg. This is comparable with that of highly purified urinary HCG but, according to the earlier mentioned studies, the activity may be even higher.

The half-life of rhCG in circulation is similar to that of urinary hCG **(Gervais et al., 2003).**

It was found that there is no evidence of difference between rhCG or rhLH and uhCG in achieving final follicular

maturation in IVF, with equivalent pregnancy rates and OHSS incidence.

According to these findings uHCG is still the best choice for final oocyte maturation triggering in IVF and ICSI treatment cycles. **(Youssef et al., 2011)**

Route of administration of hCG:

The threshold dose of HCG necessary to induce meiotic reactivation of the oocyte has been said to be between 2000 and 5000 IU **(Abdalla et al, 1987)**.

However, the threshold dose is probably also dependent on the route of administration.

In ovarian stimulation cycles, the intramuscular route for administration of hCG seems to have been accepted as the most effective route **(Crook et al, 1967).**

The purified form of hCG is usually given via the intramuscular (IM) route, but subcutaneous (SC) administration has also been described **(Carina et al., 2003)**.

The initial use of hCG intramuscularly is probably due, in part, to the fact that urinary-derived gonadotrophins have only been recommended for intramuscular administration because of their impurity [specific activity being in the order of 100-150 IU follicle stimulating hormone (FSH)/ mg protein].