Association of Basal Serum Testosterone Levels with Ovarian Response and Intracytoplasmic Sperm Injection Outcome

A thesis

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AFC:	Antral Follicular Count
AMH:	Antimullerian Hormone
AR:	Androgen Receptors
ASD:	Androsterodione
COH:	Controlled ovarian hyperstimulation
DHEA:	Dehdroepiandrosterone
FSH:	Follicular Stimulating Hormone
GnRH:	Gonadotropin Releasing Hormone
HCG:	Human Chorionic Gonadotropin
HMG:	Human Menopausal Gonadotropin
ICSI:	Intracytoplasmic Sperm Injection
IVF:	Invitro Fertiliztion
LH:	Leutinzing Hormone
TSH:	Thyriod Stimulating Hormone
T:	Testosterone

INTRODUCTION

Infertility is a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse **(Zegers-Hochschild et al., 2009).**

Infertility affects up to one in seven couples all over the world. A propotion of these couples may be able ultimately conceive , but for the majority conception is unlikely without some form of medical intervention **(Collin, 2004).**

Intracytoplasmic sperm injection (ICSI) is a laboratory procedure developed to help infertile couples undergoing in vitro fertilization (IVF) due to male factor infertility**(ASRM,2001).**

In Vitro Fertilization (IVF): is defined by the American Society Of Reproductive Medicine (ASRM) as "a method of assisted reproduction in which a man's sperm and a woman's eggs are combined outside of the body in a laboratory dish. If fertilization occurs, the resulting embryos are transferred to the woman's uterus, where one or more may implant in the uterine lining and develop **(ASRM, 2003).**

on the characteristics of the couples being treated,and the performance of the clinic. The former cannot be changed, and embryology laboratories have worked hard to optimize procedures. Numerous studies have been reported on how to improve insemination and culture procedures. The clinicians' role is confined to stimulation, oocyte collection and embryo transfer. Although each edition of journals on reproductive medicine contains some reports on various stimulation regimens, the physical aspects of oocyte collection and embryo transfer have received limited interest **(Kovacs 1990).**

Ovarian response, the recruitment and development of multiple follicles followed by gonadotropins, is a key factor for in vitro fertilization (IVF) treatment cycle. The prediction of ovarian response before undertaking the expensive IVF treatment is important. However, the predictive value of various widely used markers, such as age, antral follicle count (AFC), levels of serum inhibin B, serum anti-Müllerian hormone (AMH), basal serum follicle stimulating hormone (FSH) and estradiol (E2), basal FSH/LH **(luteinizing hormone)** ratio, still appeared inconsistent and not accurate enough. A large amount of patients may respond poorly to gonadotropins in spite of normal screening parameters **(Kwee et al.,2003).**

Androgens, primarily testosterone (T) and androsterodione, are noteworthy to enhance follicular recruitment, promote follicular growth and development **(Steckler et al.,2005).**

Recent clinical reports with encouraging results demonstrated that cotreatment with androgen, such as dehydroepiandrosterone (DHEA) and Androderm (transdermal testosterone), could increase both quantity and quality of oocytes and embryos, and improve pregnancy outcomes in women with diminished ovarian function or even premature ovarian failure **(Fábregues et al.,2009).**

AIM OF WORK

The aim of this study is to detect Basal Serum testosterone level as a predicator for ovarian response and ICSI outcome.

Research Hypothesis

The relation between basal serum testosterone with ovarian response ICSI outcome.

Research Question

Can basal serum testosterone level predict ovarian response and ICSI outcome in patient going for ICSI?

REVIEW OF LITERATURE

Physiology of the Normal Menstrual Cycle:

Ovulatory menstrual bleeding can be considered the final curtain call on a precisely choreographed play that, each month, sees hypothalamic, pituitary, and gonadal hormones interact in an effort to optimize circumstances for reproduction. In non-contracepting women, when not interrupted by pregnancy or lactation, this play can be expected to run for some 40 years (**Macklon and Fauser, 2001)**.

Estimates suggest that a century ago women would experience fewer than 100 menstrual cycles in a lifetime because of repeated interruptions for pregnancy followed by lengthy intervals of breast-feeding. In contrast, today's woman can expect to experience many more menstrual cycles in her lifetime in part due to earlier menarche, later menopause, and widespread use of effective contraception resulting in fewer pregnancies (**Macklon and Fauser, 2001)**.

The fetal ovary is reported to contain some seven million germ cells in the fifth month of gestation. Most of these are lost before birth, with the newborn female having only 1–2 million oocytes remaining. By puberty the number of oocytes has dropped to 500 000 and it is from this pool that one egg matures and an additional 1000 oocytes are lost each month until the oocyte pool is ultimately depleted and menopause begins (**Macklon and Fauser, 2001 & Richardson et al., 1987**). In the final 5 years before menopause the remaining oocytes show increasing resistance to gonadotropic stimulation, resulting in menstrual cycles which are irregular, unpredictable, and punctuated by transient signs and symptoms of menopause. Although the

phase from menarche to menopause is often referred to as 'the reproductive years' it is now clear that the competence of oocytes declines steadily with age and that pregnancy is an uncommon event after age 40 years old. In the absence of contraception or other endocrine disruption to menstrual cyclicity (e.g. excessive weight loss or gain, thyroid dysfunction, hyperprolactinemia, etc.), the modern woman can expect to have 400–450 menstrual cycles in her lifetime (**Eaton et al., 1994**).

Pubertal Onset of Hypothalamic Activity :

The hypothalamic deca-peptide gonadotropin-releasing hormone (GnRH) regulates the synthesis and secretion of the pituitary gonadotropins – luteinizing hormone (LH) and follicle-stimulating hormone (FSH) – that are ultimately responsible for ovarian activation. A hypothalamic 'pulse generator' regulates the pulsatile release of GnRH. Throughout childhood, central mechanisms block the release of GnRH. Typically around the age of 11 or 12 years old, this central restraint declines – initially resulting in nocturnal pulses of GnRH and ultimately in 24-hour GnRH pulsatile secretion. These GnRH pulses, in turn, lead to pituitary release of LH and FSH and subsequent activation of ovarian steroidogenesis (**Reid and VanVugt, 1987)** . Kisspeptins (named for the famous Hershey 'kiss' chocolate produced in the place of their discovery – Hershey, Pennsylvania) represent the most exciting new research development in our understanding of puberty (**Reid and VanVugt, 1987)**.

The kisspeptins are a family of proteins derived from the metastasis repressor gene which are ligands for GPR54, a G-protein receptor (**Lee et al., 1999**). Mutation in this receptor has been found in humans with delayed

puberty and hypogonadotropic hypogonadism (**de Roux et al., 2003 & Seminara et al., 2003**) . Administration of kisspeptin-10, the most potent ligand of GPR54, advanced pubertal onset in female rats and stimulated GnRH secretion in peri-pubertal non-human primates (**Seminara et al.,2003& Navarro, Fernandez, 2004**).

Metastasis repressor gene and GPR54 gene expression is increased in the hypothalamus of female peri-pubertal monkeys (**Shahab et al., 2005**). Together, these findings are strong evidence that GPR54 signaling is critical to the initiation of puberty (**Shahab et al., 2005**).

Ovarian Steroidogenesis :

A 'two cell – two gonadotropin' hypothesis has allowed a better understanding of the changing steroidogenesis that results during the menstrual cycle Under the influence of LH, the well-vascularized theca cells synthesize androstenedione and testosterone. These androgens diffuse through the basement membrane of the follicle to reach the avascular granulose cell layer where they form the substrate for estrogen production (**Chabbert and Bouchard, 2002**).

FSH exerts its effects primarily on the granulosa cells that line the inside of the antral follicle, causing (1) mitosis and a rapid increase in granulosa cell numbers, (2) an increase in cell surface FSH and LH receptors, and (3) the acquisition of aromatase activity by granulose cells (**Zeleznik, 2001)**. As aromatase within this follicle converts androgens to estrogen, estrogen is released into the circulation and exerts negative feedback at the hypothalamus and pituitary to inhibit FSH secretion (**Zeleznik, 2001)**.

As the menstrual cycle progresses, there is gradual reduction in FSH release. Each of these effects is critical to the process of achieving monofollicular ovulation. Follicular growth starts out as an FSH-independent process. Primordial follicle development is slow and highly variable, at times taking 120 days to develop into 2 mm pre-antral follicles. This process is almost certainly regulated by intra-ovarian peptides in a paracrine fashion**.** The final 15 days of follicular growth depends on a cyclical rise in FSH **(Chabbert-Buffet and Bouchard , 2002)**.

Follicular development in this final stage (representing the 'follicular phase' of the menstrual cycle) has been divided into three stages: 'recruitment', 'selection', and 'dominance'. The phase of 'recruitment' starts a day or two before menstruation and concludes by day 4 of the follicular phase. The onset of this phase is initiated by the demise of the corpus luteum from the preceding cycle. The hypothalamus and pituitary are released from the restraining effects of progesterone and inhibins produced within the corpus luteum, resulting in a rapid rise in FSH and the 'recruitment' of a new cohort of antral follicles to enter the maturation process. By day 5, one follicle from this cohort starts to gain a competitive advantage over the other recruited follicles**(Chabbert-Buffet and Bouchard , 2002)**.

Which follicle will be selected is probably more a matter of chance than of destiny. Analogous to pups around a food bowl – one pup, by chance, gets slightly larger than the others. This results in a competitive advantage that allows that pup to continue to gain over its litter mates **(Chabbert-Buffet and Bouchard , 2002)**.

As one follicle gets a slight 'head start' in its growth it becomes slightly more sensitive than neighboring follicles to FSH. Its competitive advantage comes from the fact that it releases into circulation increasing amounts of estrogen, that in turn suppresses the pituitary release of FSH needed to allow continued growth of smaller follicles in the cohort. Since the slightly larger follicle has acquired more granulosa cells, more FSH and LH receptors, and more aromatase activity, it is better able to survive the falling FSH levels. Recent evidence indicates that FSH and LH receptors share a common intracellular cyclic AMP pathway; hence, the 'selected' follicle can develop in response to either FSH or LH, unlike smaller follicles in the cohort, which have few LH receptors. For the remainder of the cohort, the drop in FSH at such an early stage in development results in their gradual demise by day 8 of the cycle. At the same time, the selected follicle appears to release certain proteins that have paracrine effects. Vascular endothelial growth factor (VEGF) results in an increased density of capillaries around the dominant follicle and thereby enhances delivery of FSH, LH, and steroidogenic substrates necessary for final development of the dominant follicle. Ultimately, this 'dominant' follicle undergoes exponential growth over the final 5–6 days of the follicular phase, culminating in ovulation around day **(Nippoldt et al., 1989)**.

Interplay Between Ovarian and Pituitary Hormones :

Both the frequency and amplitude of GnRH pulses change throughout the menstrual cycle in response to feedback effects of the gonadal steroids estrogen and progesterone on hypothalamic neuroregulators such as dopamine and endogenous opioid peptides.

The hypothalamic GnRH pulse generator produces one pulse per hour in the absence of ovarian restraint (**Marshall et al., 1991).** During the luteal–follicular transition when estradiol, progesterone, and inhibin levels fall, pulses of GnRH occur every 90–120 minutes. In the mid to late follicular phase, GnRH frequency increases to one pulse per hour, favoring LH secretion, while FSH is inhibited by rising levels of estradiol and inhibin B from the dominant follicle (**McCartney et al., 2002**).

Differential regulation of FSH and LH secretion late in the follicular phase is accomplished by at least two different mechanisms (**McCartney et al., 2002**). First, estradiol and inhibin A feedback to the pituitary gonadotrope to augment LH and inhibit FSH secretion (**Nippoldt et al., 1989**). Secondly, a changing (more rapid) pattern of GnRH pulses favors LH release (**Marshall and Griffin, 1993**). The LH surge onsets most often between midnight and 8 am and is unlikely to occur before the follicle diameter has achieved 15 mm and or serum estradiol reaches 600 pmol/L (**Cahill et al., 1998**).

The LH surge induces resumption of meiosis, luteinization of the granulosa cells that line the interior of the dominant follicle, and a series of inflammatory events that precipitate follicular rupture (**Tsafriri et al., 1993**). The LH surge lasts for approximately 48 hours and ovulation occurs about 36 hours after the onset and 12 hours from the peak serum LH concentration. Granulosa cells are decidualized by high concentrations of LH and acquire the ability to produce progesterone (**Buffet and Bouchard, 2001**).

After ovulation, this production of progesterone by the corpus luteum influences opioidergic, noradrenergic, and – aminobutyric acid (GABA)

systems within the hypothalamus, slowing GnRH pulse frequency to one pulse every 3–5 hours (**Buffet and Bouchard, 2001**). Corpus luteal production of estradiol and inhibin A reduces FSH release into the circulation, resulting in accumulating pituitary stores of FSH. When the corpus luteum eventually undergoes demise, the subsequent FSH release starts recruitment of a new cohort of follicles (**Marshall et al., 2001**).

LH pulses (as a marker for GnRH pulse frequency) have been examined through frequent sampling of blood at different phases of the menstrual cycle in women with premenstrual syndrome (PMS) and controls, with no significant differences being detected (**Reame et al., 1992**).

Ovarian Steroid Fluctuations During the Menstrual Cycle :

The menstrual cycle is typically described in two phases: the *follicular phase*, a 10–14 day period leading up to ovulation; the *luteal phase*, the 12–14 day phase from ovulation until the onset of menstrual bleeding. Low levels of estradiol are present from immediately prior to menstrual bleeding until the dominant follicle begins to grow (**Parrish, 2006**).

Late in the second week of the menstrual cycle, estradiol concentrations reach approximately 1000 pmol/L. Coincident with follicular rupture, there is a transient but abrupt fall in estradiol levels for a 24–48-hour period. This abrupt midcycle drop in estradiol has been associated with endometrial destabilization and bleeding in some women (**Bromberg and Bercovici, 1956).** and transient PMS-like mood symptoms in others (**Reid, 1983).** Ovulatory pain has been termed ‘mittelschmerz’ while the periovulatory mood disturbance is recorded as ‘mittelwahn’ in the older