The Influence of the Clinical Insulin Suppressing Diet on Female Infertility

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Medicine with Industrial Specialization Department of Health Science and Technology Aalborg University Project group: 1001 Project period: 3rd February to 28th May 2014 Supervisor: Linda Pilgaard External Supervisor: Bjarne Stigsby Number of pages: 31 Appendices: 0

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The Influence of the Clinical Insulin Suppressing Diet on Female Infertility

Abstract

Insulin resistance (IR) and compensatory hyperinsulinemia have been linked to conditions contributing to female infertility. The aim of this study was to investigate whether the Clinical Insulin Supressing (klinisk insulinsænkende, KISS) diet, which targets IR, improves the reproductive outcome of infertile women.

A retrospective study of infertile patients treated with either homologues semen (n=799) or donor semen (n=91) in Gynækologisk Klinik Taastrup (GKT) was performed. All patients had been prescribed the KISS diet. It was assumed that women who were hyperinsulinemic before the diet intervention were better responders than normoinsulinemic women. On the basis of this, it was hypothesized that women who became pregnant would have higher baseline C-peptide levels than women failing. In addition, the pregnancy rate in GKT for women inseminated with donor semen was compared to the national average reported by the National Danish Fertility Society. Confidence intervals (CIs) were computed and used to assess statistical significance. The spontaneous pregnancy rate for women treated with partner's semen was also compared to rates from other sources.

There was no difference in baseline C-peptide levels between pregnant and non-pregnant women, neither in women treated with partner's semen nor in women undergoing donor insemination (P>0.05). In women below 40 years of age who were inseminated with donor semen, the pregnancy rate in GKT and the national average appeared to be similar (12.3 % vs. 12.9 %). In women 40 years of age or above, the pregnancy rate in GKT of 14.9 % (95 % CI 7.4-25.7) was significantly higher than the national average of 5.7 % (95 % CI 4.8-6.7), as the CIs were not overlapping. The spontaneous pregnancy rate in women treated with husband's semen in GKT was 33.3 %, which was higher than reported by other studies, but significance could not be tested. Since the KISS diet may be the main difference between GKT and other clinics, these findings may suggest that this diet improves the fertility treatment outcome, especially in women of advancing age.

A clear limitation to this study was the lack of an appropriate control group among others. Hence, the obtained results should be interpreted with caution. Randomized controlled trials, preferentially multicenter studies, are necessary in order to clarify the effect of the KISS diet on infertility.

1. Introduction

1.1 Epidemiology

Today approximately 8 % of all children born in Denmark are conceived by medically assisted reproduction (MAR) methods (1). This bears witness of the fact that numerous couples are affected by infertility. The European Society of Human Reproduction and Embryology (ESHRE) estimates that the lifetime prevalence, i.e. the proportion of couples experiencing infertility at some point in life, is approximately 16 % worldwide (2). A higher lifetime prevalence of 26 % has been inferred from a Danish questionnaire survey (3). An alternative way of expressing the occurrence is by means of the current prevalence. In contrast to the lifetime prevalence, the current prevalence only includes women who are infertile at the time of the investigation, disregarding former episodes of infertility. The ESHRE reports that the current prevalence is 9 % at a global level (2).

1.2 Actiology of infertility

Infertility can be defined in a number of ways, but in a clinical setting it is predominantly defined as the inability to become pregnant after 12 months of regular unprotected sexual life (4,5). Infertility can be subdivided into primary and secondary infertility. Primary infertility comprises women unable to achieve a pregnancy who have never been pregnant before, whereas secondary infertility relates to women who have previously been pregnant, but have failed to become pregnant again (6).

Infertility may be attributable to female factors, male factors, or both. The American Society for Reproductive Medicine estimates that female and male problems each account for 1/3 of infertility cases, and the remaining 1/3 of cases are either idiopathic or due to a combination of both female and male factors (7).

The causes of infertility are diverse, but especially two reasons in reference to female infertility prevail, namely failure to ovulate and tubal disease (8). Ovulation and the preceding oocyte maturation are regulated and affected by a wide range of hormones, so any disruptions in these hormones may potentially cause anovulation, thus minimizing the chance of conception (9). Other underlying reasons for anovulation include premature menopause, Turner's syndrome, and luteinized unruptured follicle syndrome (9,10). The second main reason for female infertility is tubal disease. This may be a result of an infection, which induces local inflammation. Consequently, it can lead to damage or blockage of the fallopian tubes. Tubal disease is often linked to infections caused by Chlamydia trachomatis (11,12), which pose a great threat as they are highly prevalent among women of reproductive age (13) and often have an asymptomatic appearance (14). In addition to ovulatory problems and tubal endometriosis disease. and increasing maternal age may also explain female infertility (6). Causes of male infertility are most often related to semen abnormalities including oligozoospermia, azoospermia, impaired spermatozoa motility, or abnormal morphology (6).

1.3 Traditional fertility treatment options

Infertility causes some of the affected couples to seek medical attention. According to the Danish Health and Medicines Authority approximately 30,000 Danish women underwent MAR in 2010 (1). MAR should be distinguished from assisted reproductive technology (ART), although these terms are sometimes used interchangeably (4). ART includes fertility treatments involving in vitro handling of gametes for instance in vitro fertilization (IVF), but it does not include intrauterine insemination (IUI) (5). MAR, on the other hand, comprises both ART procedures and IUI (5).

The choice of fertility treatment depends on the given individual circumstances for each couple or woman. The most commonly used treatment among all in Denmark is IUI, which is selected in about 54 % of the cases (1). IUI involves placement of sperm directly into the top of the uterus and may either be performed with semen from the husband/partner, i.e. homologous (IUI-H), or a donor (IUI-D). The remaining treatment options are ART procedures, the most prevalent of these being IVF and intra-cytoplasmic sperm injection (ICSI). Together they constitute approximately 33 % of all fertility treatments performed in Denmark (1). The IVF procedure involves retrieval of oocytes to which several spermatozoa are added in order to achieve fertilization. ICSI involves in vitro injection of a single carefully selected spermatozoon into an oocyte. Alternative fertility treatment options include surgical sperm retrieval, frozen embryo replacement, and oocyte donation among others.

Drug administration during fertility treatments is common. The utilization of stimulatory drugs varies according to the selected fertility treatment. Additionally, differences between the fertility departments and clinics in Denmark exist, although general guidelines can be deduced. The following description of a general fertility treatment course is based on patient instruction sheets from various Danish clinics.

IUI-H is typically offered when infertility is caused by ovulatory problems, mild endometriosis, mildly to moderately impaired semen quality, or idiopathic reasons (6). IUI-H can both be performed with and without preceding hormonal stimulation. The hormonal stimulation involves maturation of the follicle prior to insemination. For this purpose Clomiphene citrate (Pergotime®) is first line treatment. This drug produces an increase in the gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH), released from the anterior pituitary gland (15). Clomiphene citrate is often supplemented with hormone injections (Gonal-F®, Puregon®, Menopur®) to assist the follicle maturation. Once a mature follicle can be identified on transvaginal ultrasound, ovulation is induced by abdominal cutaneous injections of an LH analogue (Ovitrelle®, Pregnyl[®]). The actual insemination is performed with purified sperm 36-38 hours later (16,17).

Couples affected by tubal disease, severely reduced semen quality, or who have had three unsuccessful IUI attempts are normally offered IVF (6). IVF implies a more extensive drug use compared to IUI. Both a long and a short protocol are available, with the long protocol being the most applied. The first step in the long protocol is medical suppression, so-called down regulation, of the natural female hormonal cycle by administration of a nasal spray (Synerela®, Suprecur®) congonadotropin-releasing taining hormone (GnRH). This step optimizes and eases controllability of the subsequent stimulation of follicle maturation, which is facilitated by hormonal injections (Gonal-F®, Puregon®, Menopur®). As in IUI, an LH analogue is used for triggering ovulation. Subsequently, oocytes are retrieved, fertilized and transferred to the uterus. Aftercare involves use of a gel containing progesterone (Crinone®) in order to achieve an optimal environment for implantation.

Thus, comprehensive drug administration is often implicated in both IUI and IVF procedures. Adverse drug effects such as abdominal pain, nausea, bloating, mood and changes, ovarian hyperstimulation syndrome (OHSS) are common (18). An alternative or supplementary approach aims at improving insulin resistance (IR), which may play a key role in both male and female infertility. Male infertility is not the scope of this project, and therefore only the impact of IR on female fertility will be outlined in the following.

1.4 Insulin resistance in female infertility

IR is a condition in which the response to insulin is decreased, thereby hampering the uptake of glucose in the cells. Consequently, hyperglycemia may arise. To compensate for this, pancreatic β -cells increase their secretion of insulin causing a hyperinsulinemic state. IR is considered to be a hallmark of diabetes

mellitus type II (19), but it is also frequently found in obese individuals (20,21) and women with polycystic ovary syndrome (PCOS) independent of obesity (22). Further, it has also been associated with conditions such as stress (23,24), sedentary lifestyle (25), advancing age, (26,27) and hypertension (28,29). Accumulating evidence suggests that IR is also implicated in female infertility, as hyperinsulinemia is related to unfavourable conditions for establishing a pregnancy. Firstly, hyperinsulinemia has been associated with increased androgen levels. Secondly, it has been demonstrated that oocyte quality and embryonic development are impaired in hyperinsulinemic states. Thirdly, endometrial function is possibly negatively affected by hyperinsulinemia. These three consequences are elaborated in the following paragraphs.

1.4.1 Association between hyperinsulinemia and hyperandrogenism

In various conditions, hyperinsulinemia and concurrent hyperandrogenism are found. Rare examples include disorders such as leprechaunism and Rabson-Mendenhall syndrome (30,31), but hyperinsulinemia coexisting with hyperandrogenism is also a common finding in obesity (21) and polycystic ovary syndrome (PCOS) (32,33). Moreover, several studies have shown that administration of an insulin-sensitizing agent such as metformin (34-36) or troglitazone (37,38) reduces hyperandrogenism in PCOS patients. All together, these findings support the existence of a link between hyperinsulinemia and hyperandrogenism.

The poor reproductive outcome in hyperandrogenemic states is probably linked to menstrual cycle irregularities and anovulation, since a study by Steinberger et al. (39) demonstrated that increased testosterone levels in infertile women correlated with amenorrhea and anovulation.

Hyperinsulinemia is thought to produce elevated androgen levels because insulin stimulates ovarian steroidogenesis (40). LH may be involved in this effect, since studies have demonstrated that insulin and LH act synergistically to enhance steroidogenesis (41,42). An alternative explanation for the insulin-mediated hyperandrogenism may be that insulin affects enzymes involved in steroidogenesis. In PCOS patients an insulinmediated increase in the activity of ovarian cytochrome P450c17 α , which is an enzyme implicated in androgen synthesis, has been proposed (43,44). Conflicting results were obtained by Unluhizarci and co-workers (45), who did not find metformin to alleviate the overactivity of cytochrome P450c17 α in PCOS patients.

It may seem paradoxical that the ovaries remain sensitive to insulin when the primary target tissues are resistant. An explanation for this may involve the insulin-like growth factor 1 (IGF-1) receptor. Binding of the ligand IGF-1 facilitates ovarian steroidgenesis (46). Insulin preferentially binds to the insulin receptor, but at high concentrations insulin may also bind to the IGF-1 receptor and stimulate steroidogenesis (30,47). Hence, in states of IR and compensatory hyperinsulinemia, the augmented androgen synthesis may be due to an interaction between insulin and the IGF-1 receptor. However, this mechanism does not explain all cases. Willis and colleagues (48) found that antibodies directed against insulin reduced steroidgenesis of granulosa cells from PCOS patients. Antibodies against the IGF-1 receptor, on the other hand, had no influence on steroidogenesis. This suggests that binding of insulin to its own receptor and not the IGFreceptor causes the insulin-mediated 1 steroidogenesis in PCOS patients. Nestler et al. (49) found similar results using theca cells

from PCOS patients. In addition they proposed an alternative mechanism explaining hyperandrogenism in insulin resistant states. Insulin mainly mediates its functions via tyrosine kinase signalling pathways (50), but the study revealed that alternative pathways involving inositol glycane mediators may set in when the tyrosine kinase pathway is defect.

In addition to stimulating steroidogenesis, insulin also influences sex hormone-binding globulin (SHBG) levels. SHBG is produced in the liver and binds testosterone among others, thereby reducing the fraction of biologically active hormone. A study by Nestler et al. (51) investigated the effect of insulin on SHBG concentrations in obese PCOS patients. Pancreatic insulin secretion was inhibited by administration of diaxozide, resulting in increased levels of SHBG compared to baseline. This indicates that hyperinsulinemia reduces circulating SHBG concentrations, thereby increasing the bioavailability of testosterone. This finding is supported by demonstrating a negative other studies correlation between insulin and SHBG levels in non-diabetics (52), and between SHBG and homeostasis model assessment (HOMA) values (53), which provide a measure of the IR, in women without PCOS.

1.4.2 Poor oocyte quality and impaired embryonic development in insulin resistant states

Impaired oocyte quality and embryonic development may also contribute to the poor reproductive outcome in hyperinsulinemic states. This association has been demonstrated in both animal and human studies. Ou and colleagues (54) divided mice into three groups. Group I received insulin injections to create hyperinsulinemia, group II was given injections of insulin and human chorionic gonadotropin (hCG) to induce hyperinsulinemia and hyperandrogenism, and group III comprised control mice receiving only saline injections. It was demonstrated that the ovulation rate and the number of retrieved oocytes were reduced in group I and group II compared to controls. Further, 14 % and 16 % of the oocytes had abnormal morphology in group I and group II, respectively. This was significantly higher than the 7 % of group III. Subsequently, abnormal oocytes were sorted out and the remaining morphologically normal oocytes were fertilized in vitro. It was revealed that group I and group II embryos displayed impaired development, including reduced fertilization, cleavage, and blastocyst rates. Taken together, these results indicate that IR influences oocyte quality and early embryonic development. In the same study, increased oxidative stress and mitochrondrial dysfunction were demonstrated to be possible underlying mechanisms affecting oocyte quality.

Another study (55) also using a mouse model induced obesity with a high fat diet. Administration of the insulin sensitizer rosiglitazone to a subgroup of obese mice improved embryonic development, which was evaluated at three different stages. In addition rosiglitazone significantly decreased glucose and insulin levels compared to obese mice given vehicle. These results suggest that oocyte development is influenced by insulin levels, thus supporting the findings by Ou et al. (54). The effect of another insulin sensitizer, sodium salicylat. was also same study. investigated in the Like rosiglitazone, sodium salicylat significantly decreased insulin levels. However, sodium salicylat did not reverse the obesity-induced impairment of the embryonic development. This suggests that the specific target of rosiglitazone, the peroxisome proliferatoractivated receptor-y, may play an essential role in oocyte development. In contrast to

sodium salicylat, rosiglitazone affects both carbohydrate and lipid metabolism. Although not statistically significant, a tendency of rosiglitazone, but not sodium salicylat, to lower triglyceride levels was observed in the study. This may indicate that lipid metabolism in addition to carbohydrate metabolism influences oocyte quality (56).

A human study conducted by Cano et al. (57) investigated endocrine characteristics of a group of infertile women with polycystic ovaries. The infertile women all participated in an IVF program and all provided oocytes for donation. After a two-year follow-up, the women were divided into two groups according to IVF treatment outcomes. Unsuccessful outcome was characterized by failed implantation in own and/or recipient's uterus. Women with unsuccessful outcome, and thus poor oocyte quality, were included in group I. When women in group I became recipients of donor oocytes after the failed IVF treatment, pregnancy was achieved in the first try, confirming that these women had poor oocyte quality. The oocyte quality in group II was considered to be normal, because implantation was established in own and/or recipient's uterus. Eumenorrheic women without polycystic ovaries constituted the control group. The endocrine profile of the three groups did not differ as both androgen and gonadotropin levels were similar. However, an oral glucose tolerance test (OGTT) revealed that women in group I had significantly higher glucose and insulin levels compared to group II and group III, indicating IR. Additionally, the fertilization rate in group I was significantly lower compared to group II and group III. These results point to that IR is involved in poor oocyte quality.

Based on the aforementioned studies, it is likely that IR and hyperinsulinemia contribute to impaired oocyte quality and embryonic development. The exact mechanisms still remain to be elucidated, but causes including oxidative stress in endometrial cells and mitochondrial dysfunctions have been proposed (54).

1.4.3 Compromised endometrial function in insulin resistant states

The risk of spontaneous abortion is increased in both PCOS (58-61) and obese women (62) in whom IR is a common finding (22,32,63-65). It has been shown that metformin treatment during pregnancy reduces the risk of early pregnancy loss in PCOS women, while simultaneously decreasing insulin levels (66,67). On the basis of these findings, it appears that IR may be accountable for the increased risk of miscarriage, bearing in mind that other factors also play a role (68,69). The causal relationship between IR and the increased rate of miscarriage may be attributable to the adverse effects of hyperinsulinemia on endometrial function. This effect of hyperinsulinemia was investigated in a study by Jacubowicz and colleagues (70). They found the levels of glycodelin and insulin-like growth factor-binding protein 1 (IGFBP-1) to be substantially higher in PCOS patients receiving metformin compared to placebo. Glycodelin, also called placental protein 14, is secreted by the endometrium (71) and one of its important functions is to create a proper uterine milieu for pregnancy, possibly through suppression of an immunological response against the embryo (72,73). IGFBP-1 is also implicated in endometrial function as it is thought to enhance the embryonic implantation (74). The increased levels of glycodelin and IGFBP-1 mediated by metformin were concurrent with reduced levels of insulin. In addition, uterine vascularity and blood flow were increased after metformin treatment, thereby promoting an environment optimal for implantation and

sustained pregnancy. These results indicate that hyperinsulinemia has a negative impact on endometrial function. However, these authors also reported reduced androgen levels in the metformin group compared to placebo. Thus, as emphasized by the authors, it cannot be ruled out that the observed effects were mediated at least partially by reduced androgen levels, since elevated androgen levels also have been connected to spontaneous abortions (75,76).

1.5 An alternative approach to treatment of female infertility

Based on the above, it appears that IR and hyperinsulinemia adversely affect female fertility via disruption of the hormonal environment, reduced oocyte quality, impaired embryonic development, and compromised endometrial function. Accordingly, non-pharmacological treatment aiming at ameliorating IR and hyperinsulinemia may improve fertility outcome in addition to insulin-sensitizing agents. Studies have demonstrated that weight loss and physical activity enhance insulin sensitivity in overweight and obese subjects (77-80). In addition a proper diet may also reduce IR and hyperinsulinemia, thereby potentially restoring fertility.

1.5.1 The Clinical Insulin Suppressing Diet for infertility

A specific diet targeting IR was invented by the Danish gynaecologist Bjarne Stigsby. This diet called the Clinical Insulin Suppressing (klinisk insulinsænkende, KISS) diet operates with an even tripartition of the dietary energy intake, meaning that about 1/3 of the energy should be derived from carbohydrate, 1/3 from protein, and 1/3 from fat (81,82). Hence, compared to the Nordic Nutrition Recommendations (83), the intake of carbohydrates should be reduced and replaced by a higher protein intake, whereas the fat intake should be maintained approximately at the same level, see figure 1.



Figure 1. Macronutrient distribution according to the Clinical Insulin Suppressing diet and the Nordic nutrition Recommendations 2012 (83).

In this diet it is essential to consume the right types of carbohydrates in order to avoid fluctuations in blood glucose levels. For this purpose, the glycemic index (GI) is valuable because it ranks different foods according to their impact on blood glucose levels.

To determine GI, test subjects ingest portions containing 50 g of available carbohydrate, and subsequently blood glucose levels are measured at certain intervals for two hours postprandial. The increase in blood glucose levels caused by either glucose or white bread containing 50 g of available carbohydrates serves as a reference and has a GI of 100 (84). High-glycemic foods are quickly digested and absorbed from the intestines, leading to rapid elevations in blood glucose and insulin levels. The greater the increase in blood glucose levels, the higher the risk that insulin secretion exceeds the need and lowers the blood glucose levels below the normal range (85). Low-glycemic foods only give rise to minor increases in blood glucose and insulin levels, and therefore they should constitute the majority of the carbohydrates in the KISS diet.

The rate at which different types of carbohydrates affect blood glucose levels vary, and this is reflected in the GI. However, GI does not take the quantity of carbohydrates in a food into account, but the glycemic load (GL) does. GL is calculated on the basis of a food's GI multiplied by its available carbohydrate content in a portion size of 100 g divided by 50 (81,82).

Because foods with high GI and/or GL are both disadvantageous, Bjarne Stigsby created the sugar index (SI). A food's SI is equal to the highest value, either the GI or GL (81,82). Based on the SI, foods are categorized into three groups, indicating how often they should be consumed. Foods with a SI<40 should constitute most of the daily caloric intake, whereas foods with a SI between 40 and 55 should be consumed in moderation. Foods with a SI >55 should be avoided or only rarely consumed.

2. Aim

The aim of this thesis was to investigate if the KISS diet improves the reproductive outcome of infertile women. All patients had been prescribed the diet and were retrospectively divided into two groups according to whether or not they became pregnant. It seems reasonable to assume that women with high baseline insulin levels would benefit the most from the diet compared to women who at baseline were normoinsulinemic, because hyperinsulinemic women have the greatest improvements potential for with diet modification. On the basis of this, it was hypothesized that women achieving pregnancy would have significantly higher baseline insulin levels than women not achieving pregnancy. In order to further investigate the aim of this study, the pregnancy and spontaneous pregnancy rates were examined and compared to rates reported by other published sources. This type of comparison was performed, because all women included in this study had been encouraged to follow

the diet, and therefore no proper control group was available.

3. Materials and methods

3.1 Patients

From August 2006 to January 2013, a total of 984 infertile patients started treatment with partner's semen at Gynækologisk Klinik Taastrup (GKT). Of these 799 patients were included in this study. Patients were retrospectively divided into two age groups; <40 and ≥ 40 years of age. Women in each age group were then subdivided into two additional groups according to whether or not pregnancy was established and maintained at least until the 12th gestational week. Figure 2 shows a flow diagram of the patients. The mean age and the mean body mass index (BMI) of the patients were 31.5±5.2 years and 25.8 ± 5.9 kg/m², respectively. Patients were excluded from the study in case of an unregistered pregnancy outcome. An unregistered outcome was either due to erroneously missing values or an unknown outcome, because treatment was still in progress when the data collection was terminated. It was not possible to distinguish between these two causes. Three patients were excluded because it was impossible to

conclude whether or not pregnancy was achieved, as data were ambiguous. Data for all patients had been compiled in a database, which was anonymized, and therefore no approval from the regional ethics committee or the Danish Health and Medicines Authority was required prior to this study. The database was searched for errors and conflicting data. In case of errors or conflicting data the implicated values were set as missing. Patients with a missing value for a given variable were not included when calculating percentages.

115 women began IUI-D treatment at GKT between May 2010 and April 2014. A total of 91 patients were included, whereas 24 of the 115 patients were excluded from the analysis either because of an unregistered pregnancy outcome or missing age, see figure 3. The division of patients into groups was based on the same conditions as described above. The mean age of the women was 37.0 ± 4.8 years, whereas the mean BMI was 24.3 ± 4.4 kg/m². Errors and conflicting values in the dataset were set as missing. When proportions were calculated, patients with a missing value for a given variable were excluded.



Figure 2. Flow diagram of patients in treatment with partner's semen

3.2 Treatment protocol

Both women attempting to achieve pregnancy with partner's semen and women who were inseminated with donor semen were instructed in the principles of the KISS diet and were recommended to comply with these principles for 2-4 months. In addition, some patients received 500-850 mg of metformin 2-3 times daily. Commencement of metformin treatment was based on an individual assessment, taking previous miscarriages, Cpeptide level, and expected compliance to KISS diet into consideration. If no spontaneous pregnancy occurred within the 2-4 month period, hormone therapy and/or IUI-H treatment were initiated. Hormone therapy involved administration of clomiphene citrate (Pergotime®) at a dose of 50-100 mg daily from cycle day 3-7 and FSH (Gonal-F®, Puregon®) at a dose of 37.5-50 IU/day from cycle day 8-10. At cycle day 12 ultrasonography was performed and an triggering ovulation adinjection was ministered if the dominant follicle measured more than 17 mm in diameter. Timed intercourse or IUI was accomplished 36 hours later.

3.3 Study parameters

The anthropometric data included age and BMI. BMI was calculated as the weight measured in kilos divided by the square of the height in metres. Study parameters describing treatment characteristics comprised treatment time and use of additional fertility promoting initiatives besides the dietary intervention including metformin administration, hormone treatment, and use of IUI. In addition the number of IUI attempts were registered. For women treated with partner's semen, 358 patients of 799 (44.8 %) used metformin, 242 patients (30.4 %) received hormones, and 342 patients (42.8 %) underwent IUI-H treatment. For women undergoing donor insemination, 29 out of 91 patients (28.6 %) were treated with metformin and 46 patients (50.5 %) were treated with hormones.

Concentrations of baseline C-peptide were used to assess the degree of IR, since Cpeptide levels reflect the secretion of insulin. C-peptide connects the A- and B-chain of insulin, all together constituting the precursor proinsulin, and it is released in a 1:1 ratio with insulin. High baseline C-peptide levels were used as a surrogate of IR, since a study



Figure 3. Flow diagram of patients inseminated with donor semen

demonstrated that insulin resistant subjects had higher levels of C-peptide than noninsulin resistant subjects (86). C-peptide levels were obtained after an overnight fast before the diet intervention was initiated.

The pregnancy rate per cycle was determined for women who underwent IUI-H and all women in IUI-D treatment. It was calculated as the total number of pregnancies divided by the total number of cycles. For all women, the rate of spontaneous abortions was investigated, and it was calculated by dividing the total number of abortions with the total number of pregnancies. The rates of singleton, twin and triplet pregnancies were calculated by using the total number of women who achieved pregnancy in the denominator.

3.4 Statistical analysis

Either a two-sample t-test or a Mann-Whitney U-test was performed to examine differences between the pregnant and non-pregnant group for metric variables. For categorical variables the Chi-square test was used for analysis. Predictors of achieving pregnancy were identified by multivariate logistic regression, using the enter method in which all independent variables were included in a single step. Investigated explanatory variables included age, BMI, and C-peptide levels. Computation of confidence intervals (CIs) was based on the Clopper-Pearsons method. Results are presented as means and the standard deviation of the mean (mean±SD) or proportions. The statistical analysis was performed in SPSS version 22.0. P-values <0.05 were considered statistical significant.

4. Results

4.1 Patients treated with partner's semen

A total of 799 infertile patients, who completed fertility treatment in GKT, were included in the analysis of this study. Of these 733 patients were below 40 years of age, and 66 patients were 40 years of age or above. In the youngest age group, 48.2 % of the patients achieved a sustained pregnancy during the treatment course, whereas 51.8 % did not become pregnant or miscarried within the 12th week of gestation. In women 40 years of age or above, 16.7 % fell into the pregnant group and 83.3 % fell into the non-pregnant group.

Anthropometric and biochemical parameters as well as characteristics of the fertility treatment in both age groups were investigated, and the results are displayed in table 1. Among women aged younger than 40 years, age and BMI were similar between the pregnant and non-pregnant group. The time in treatment (P=0.006), the proportion of patients treated with hormones (P<0.001), the proportion of patients undergoing IUI-H (P=0.011), and the mean number of IUI-H cycles (P<0.001) were significantly higher in the non-pregnant group compared to the pregnant group. No significant difference in the proportion of patients receiving metformin was observed between the two groups. The baseline C-peptide levels were mean 630.4±270.1 pmol/L and 640.8±322.4 pmol/L among pregnant and non-pregnant women, respectively, and no statistical significant difference was detected (P=0.882). Among women aged 40 years or older, all variables including age, BMI, time in treatment, metformin administration, use of IUI-H, number of IUI-H cycles, and C-peptide levels were similar between pregnant and nonpregnant patients. The only exception was the proportion of women who received hormones, which was significantly higher among nonpregnant women (P=0.005).

Of 796 women, 265 (33.3 %) conceived spontaneously, and in 96 (12.1 %) of the patients, pregnancy occurred as a result of IUI-H (data were missing for three women; results are not shown).

The pregnancy rate per cycle for those pa-

	Age<40 years (<i>n</i> =733)			Age≥40 years (<i>n</i> =66)		
	Pregnant	Non- pregnant	<i>P</i> -value	Pregnant	Non- pregnant	<i>P</i> -value
Age (years)	30.2±4.3	30.9±4.5	0.054	40.9±0.8	41.7±1.5	0.113
BMI (kg/m ²) ^a	26.1±6.0	25.6±5.9	0.115	23.1±1.3	25.0±5.1	0.490
Treatment time (days) ^b	195.7±146.0	233.4±169.6	0.006*	200.2±110.8	250.9±188.9	0.668
Metformin (%)	48.4	42.6	0.114	36.4	38.2	0.910
Hormone (%)	21.2	37.6	<0.001*	0	45.5	0.005*
IUI-H (%)	36.8	46.1	0.011*	45.5	58.2	0.438
No. of IUI-H attempts	0.74±1.2	1.56±2.08	<0.001*	0.73±1.0	2.07±2.3	0.121
C-peptide (pmol/L) ^c	630.4±270.1	640.8±322.4	0.882	536.5±202.6	615.0±356.3	0.757

Table 1. Anthropometric, treatment, and biochemical characteristics of pregnant and non-pregnant women treated with partner's semen. Data are presented as means±SD or as proportions. BMI, body mass index; IUI-H, intrauterine insemination with homologous semen.

* Indicates a statistical significant difference between the groups (P < 0.05)

^a 4 missing values for age<40 years and 1 missing value for age \geq 40 years

 $^{\rm b}$ 3 missing values for age<40 years and 1 missing value for age≥40 years

^c 1 missing values for age<40 years

	Age<40 years	Age≥40 years
Pregnancy rate per cycle (%)	20.1	6.6
Abortions (%) ^a	16.2	26.7
Pregnancies ^b — Singletons (%)	97.7	100
Twins (%)	2.0	0
Triplets (%)	0.3	0

Table 2. Pregnancy and abortion rates, and the distribution of singleton, twin, and triplet pregnancies. Data are presented as proportions. Only women undergoing intrauterine insemination with homologues semen (n=342) and not the full sample were included when calculating the pregnancy rate per cycle.

^a 11 missing values

^b 7 missing values

Independent variable	<i>P</i> -value	OR	95 % CI
Age	<0.001*	0.936	0.910-0.962
BMI	0.096	1.025	0.996-1.056
C-peptide	0.108	1.000	0.999-1.00

Table 3. Multiple logistic regression analysis with pregnancy outcome as dependent variable. All women were included in the analysis (*n*=793, 6 missing values). BMI, body mass index; OR, odds ratio; CI, confidence interval.

* Indicates a statistical significant association between the dependent and independent variable (P<0.05)

tients who underwent IUI-H was 20.1 % and 6.6 % for women aged <40 and \geq 40 years, respectively. The results are shown in table 2. The abortion rate among all women treated with partner's semen was 16.2 % for women younger than 40 years and 26.7 % for women aged 40 years or older. The rates of singleton, twin, and triplet pregnancies in women younger than 40 years were 97.7 %, 2.0 % and 0.3 %, respectively. Among pregnant women aged 40 years or older, all had singleton pregnancies.

Predictors of successful pregnancy outcome were identified by a multivariate logistic regression analysis, and the results are shown in table 3. The only variable reaching statistical significance was age (odds ratio (OR): 0.936, P<0.001). Increasing age was associated with decreased chance of pregnancy since the OR was less than 1.

4.1.1 Comparison of pregnancy rates for patients treated with partner's semen

Each year the National Danish Fertility Society (NDFS) publishes a report on fertility treatment results in Denmark. These reports are based on mandatory reporting by public and private fertility clinics to the Danish Health and Medicines Authority. In the following, pregnancy rates from GKT and the NDFS were compared because an appropriate control group was lacking in this study.

As mentioned above, the pregnancy rates in GKT were 20.1 % (95 % CI 17.5-23.0) and 6.6 % (95 % CI 2.9-12.5) in women <40 and \geq 40 years of age, respectively. According to the NDFS, the pregnancy rate for women aged below 40 years was 12.8 % (95 % CI 12.1-13.5), whereas the pregnancy rate for women aged above 40 years was 5.0 % (95 % CI 3.5-6.9), see figure 4. Thus, the pregnancy rate in the youngest age group was significantly higher in GKT compared to the national average, as the CIs were not overlapping. There did not seem to be any difference in the pregnancy rates for patients aged 40 years or older.

4.2 Patients undergoing insemination with donor semen

During the inclusion period, 91 patients were enrolled in the IUI-D treatment program of



Figure 4. Pregnancy rates and 95 % confidence intervals (CIs) from Gynækologisk Klinik Taastrup (GKT) and the National Danish Fertility Society (86) among women treated with partner's semen.

* Indicates non-overlapping 95 % CIs for the proportion of women becoming pregnant in GKT and the national average

which 62 patients were under 40 years of age, and 29 patients were 40 years of age or over. In women aged younger than 40 years, 24.2 % fell into the pregnant group and 75.8 % fell into the non-pregnant group. In women aged 40 years or older, 24.1 % became pregnant and 75.9 % did not.

Anthropometric, treatment, and biochemical features of the groups are presented in table 4. There were no statistically significant differences in age or BMI between women who became pregnant and women who failed in either age group. Pregnant patients belonging to the youngest age group were more likely to have received metformin (P=0.011) compared to non-pregnant patients, but this was not observed in women aged 40 years or above. Other treatment characteristics including time in treatment, utilization of hormones, and the number of IUI-D attempts

	Age<40 years (<i>n</i> =733)			Age≥40 years (<i>n</i> =66)		
	Pregnant	Non- pregnant	<i>P</i> -value	Pregnant	Non- pregnant	<i>P</i> -value
Age (years)	33.9±4.4	39.9±3.7	0.486	42.1±1.3	42.2±1.8	0.980
BMI (kg/m²)	24.4±3.4	25.0±5.0	0.889	23.5±3.5	23.2±3.4	0.819
Treatment time (days) ^a	198.2±70.4	259.6±203.9	0.761	215.9±174.1	215.9±122.9	0.387
Metformin (%)	6.7	42.6	0.011*	14.3	18.2	0.812
Hormone (%)	53.3	48.9	0.767	42.9	54.5	0.590
No. of IUI-D attempts	2.80±1.5	2.57±2.6	0.282	2.43±1.9	2.27±1.9	0.862
C-peptide (pmol/L)	526.4±164.7	634.8±198.9	0.061	669.9±336.2	510.4±179.8	0.566

Table 4. Anthropometric, treatment, and biochemical characteristics of pregnant and non-pregnant women inseminated with donor semen. Data are presented as means±SD or as proportions. BMI, body mass index; IUI-D, intrauterine insemination with donor semen.

* Indicates a statistical significant difference between the groups (P<0.05)

^a 1 missing value for age≥40 years

	Age <40 years (<i>n</i> =62)	Age ≥40 years (<i>n</i> =28)
Pregnancy rate per cycle (%)	12.3	14.9
Abortions (%)	25.0	30.0
Pregnancies – Singletons (%)	93.3	100
Twins (%)	6.7	0
Triplets (%)	0	0

Table 5. Pregnancy and abortion rates, and the distribution of singleton, twin, and triplet pregnancies in women aged <40 and ≥ 40 years. Data are presented as proportions.

were similar in pregnant and non-pregnant women in both age groups. C-peptide levels were not significantly different between the groups.

The pregnancy rate per IUI-D cycle for women younger than 40 years of age was 12.3 % and 14.9 % for women aged 40 years or older, see table 5. Of all pregnancies 25.0 % and 30.0 % ended in spontaneous abortions in women aged <40 and \geq 40 years, respectively. The allocation of singleton, twin, and triplet pregnancies was 93.3 %, 6.7 % and 0 % for women under 40 years of age. Women aged 40 years or over all had singleton pregnancies.

4.2.1 Comparison of pregnancy rates for patients inseminated with donor semen

In women younger than 40 years of age, the pregnancy rate in GKT of 12.3 % (95 % CI 7.7-18.3) was comparable to the national average of 12.9 % (95 % CI 12.2-13.7) reported by the NDFS. The results are displayed in figure 5. Contrary, in women aged 40 years or older, the pregnancy rate in GKT was 14.9 % (95 % CI 7.4-25.7), thus significantly higher than the national pregnancy rate of 5.7 % (95 % CI 4.8-6.7).

Pregnancy rates for women undergoing IUI-D in GKT, as well as live birth rates in the United Kingdom reported by the Human Fertilisation and Embryology Authority





* Indicates non-overlapping 95 % CIs for the proportion of women becoming pregnant in GKT and the national average

(HFEA)¹ at six different age intervals were also compared (87). A comparison of the pregnancy rate in the 12th gestational week and the live birth rate was considered to be plausible, since studies have demonstrated that the risk of miscarriage is only 0.7-1.5 %in the 12th gestational week (88,89). The rates in GKT and in the United Kingdom appeared to be rather similar in women aged below 35 years (11.5 % (95 % CI 4.7-22.2) vs. 15.0 % (95 % CI 13.3-16.8)) and in women aged 35-37 years (11.7 % (95 % CI 4.8-22.6) vs. 11.4 % (95 % CI 9.3-13.7)), see figure 6. In women aged 38-39 years, there was a tendency towards a higher success rate in GKT compared to in the United Kingdom

(14.3 % (95 % CI 5.4-28.5) vs. 8.2 % (95 % CI 6.2-10.7)), but the CIs were over-lapping, so it was not possible to determine, whether or not the difference was statistically significant. In patients aged 40-42 years, the pregnancy rate in GKT of 20.0 % (95 % CI 8.4-36.9) was significantly higher compared to the live birth rate reported by HFEA, which was 5.9 % (95 % CI 4.1-8.2). In women aged 43-44 years, the rate was considerably higher in GKT than in the United Kingdom (11.5 % (95 % CI 2.5-30.2) % vs. 0.7 % (95 % CI 0.02-3.8)), but the CIs were overlapping. In women aged above 44 years or older, the rates in GKT and in the United Kingdom were both 0 %.



Figure 6. Pregnancy rates and live birth rates according to age among women inseminated with donor semen. Blue squares represent pregnancy rates for women treated in Gynækologisk Klinik Taastrup (GKT). Red squares represent live birth rates for British women reported by the Human Fertilisation and Embryology Authority (87). 95 % confidence intervals (CIs) are also illustrated.

* Indicates non-overlapping 95 % CIs for the proportion of women becoming pregnant in GKT and in the United Kingdom

¹ The report from 2010 was used for comparison, because patients in the latest report from 2011-2012 had been subdivided according to whether or not hormonal treatment was used. Using the same subdivision of patients in this study would have resulted in undesirably small groups.

5. Discussion

5.1 More comprehensive treatment of non-pregnant women

In the present study, the treatment time for women treated with partner's semen under the age of 40 years was significantly longer in patients failing to achieve pregnancy compared to patients who succeeded. Furthermore, IUI-H and hormones were more extensively used in the non-pregnant group. Hence, receiving a more comprehensive treatment did not entail an increased chance of becoming pregnant. One possible explanation is that the infertility challenge varies from couple to couple; some patients only require little treatment to become pregnant because of good preconditions, whereas others require extensive treatment necessarily becoming without pregnant because of poor preconditions.

Likewise, in women aged 40 years or above, the proportion receiving hormones was significantly higher in the non-pregnant group compared to the pregnant group. Tendencies towards longer time in treatment and more IUI-H attempts were also observed, but neither reached statistical significance. The lack of significance was possibly due to the relatively small sample sizes, especially in the pregnant group in which only 11 subjects were included.

5.2 Baseline C-peptide levels are not associated with reproductive outcome

A study by Jinno et al. (90) investigated the effect of metformin on the fertility treatment outcome of infertile women without PCOS undergoing IVF or ICSI. Approximately 30 % achieved an ongoing pregnancy with metformin administration, and it was further shown that these women had higher baseline

values of HOMA and fasting immunoreactive insulin (FIRI) compared to women who were unable to conceive. These findings suggest that women with reduced insulin sensitivity are better responders of metformin treatment. This is in line with the hypothesis of this study, stating that women achieving a pregnancy are more insulin resistant, i.e. have significantly higher baseline C-peptide levels, than those not achieving a pregnancy. This hypothesis was made because all women were prescribed the KISS diet, and thus no proper control group was available. However, no significant difference in C-peptide levels were found between the women becoming pregnant and the women failing, neither in women treated with partner's semen nor with donor semen. Concordantly, the C-peptide level was not significantly associated with the reproductive outcome in the multiple logistic regression analysis.

According to a power calculation, 36 subjects in each group were required in order to detect a difference of 200 pmol/L between the pregnant and non-pregnant groups, when α was set to 0.05, β to 0.20, and the SD to 300 pmol/L. Hence, in some groups, the sample size may have been too small to provide sufficient power to detect a difference if one existed. However, there was not even a tendency towards increased C-peptide levels in the pregnant groups, and it therefore seems unlikely that insufficient statistical power caused the insignificant results. An explanation may be that the baseline C-peptide level was the only variable available in the database, suitable for evaluation of insulin sensitivity. In clinical studies, a single measurement of the C-peptide level is normally not used for this evaluation. The gold standard to assess insulin sensitivity is the hyperinsulinemic-euglycemic clamp technique, but because it is time-consuming, impractical to perform, and has high costs,

alternative methods are often preferred. These include HOMA, the OGTT, the insulin tolerance test (ITT), or continuous infusion of glucose with model assessment (CIGMA) among others. All of these methods correlate fairly well with the gold standard technique (91-95). Perhaps a difference in insulin sensitivity between pregnant and nonpregnant women had been detected if another and more reliable measure of the insulin sensitivity had been obtainable.

5.3 Comparison of pregnancy rates

In this study, the pregnancy rate per cycle for women undergoing IUI-H aged below 40 years was significantly higher than the national average (96) (20.1 % (95 % CI 17.5-23.0)) vs. 12.8 % (95 % CI 12.1-13.5)). Statistical significance was ascertained by non-overlapping CIs. The pregnancy rate in this study was expected to be the same as the national average or even lower, because the patients selected for IUI-H presumably had greater difficulties becoming pregnant than patients in whom IUI-H was not used. This was assumed because patients undergoing IUI-H did not achieve spontaneous pregnancy within the 2-4 months of diet intervention, and patients who did not become pregnant more frequently underwent IUI-H treatment. In women 40 years of age or older, the pregnancy rate in GKT seemed to correspond to the national average as anticipated (6.6 %)vs. 5.0 %).

The reproductive outcome in women who had IUI-D performed was also investigated. Among women below 40 years of age, the pregnancy rate per cycle found in this study was 12.3 %, whereas the national average for the same age group was 12.9 % (96). Thus, the pregnancy rates seemed similar. In women aged 40 years or above, the pregnancy rate was 14.9 % (95 % CI 7.4-25.7), hence significantly higher than the national average of 5.7 % (95 % CI 4.8-6.7) as the CIs did not overlap. The impact of varying semen quality on pregnancy outcome is eliminated when using donor semen. By exclusion of the male factor, the foundation for comparing the results from different clinics is enhanced in proportion to a comparison between women treated with partner's semen. The main difference between GKT and other fertility clinics may be the recommended KISS diet. Therefore, higher pregnancy rates in GKT may indicate that the KISS diet improves the fertility treatment outcome. Women above 40 years of age appeared to be good responders of the diet. Indeed, it had been preferable to make comparisons of the observed pregnancy rates with an appropriate control group who was not prescribed the KISS diet, but this was not an option in this study.

Metformin administration is routinely used in GKT, and it cannot be ruled out that this may also have resulted in the increased observed pregnancy rates in certain subgroups. Studies in PCOS patients have shown that metformin facilitates regular menstrual cycles and improves pregnancy rates (97-99), but results of other studies are contradictory (34,100-102). Another factor which may have influenced the results is patient compliance. Patients were only advised to follow the KISS diet, but no consecutive initiatives to ensure or assess dietary compliance were carried out. Implementation of dietary assessment would possibly have encouraged some patients to stick to their prescribed diets, maybe resulting in even higher pregnancy rates.

The pregnancy rate for women inseminated with donor semen from GKT and the live birth rate in the United Kingdom reported by HFEA (87) were compared. It appeared that the results from GKT and HFEA were fairly similar in the age groups <35 years and between 35-37 years. In the age groups 38-39

years (14.3 % vs. 8.2 %), 40-42 years (20.0 % vs. 5.9 %) and 43-44 years (11.5 % vs. 0.7 %), the chance of a successful pregnancy outcome was higher in GKT compared to in the United Kingdom, but only in women aged 40-42 years this difference was statistically significant as the CIs were not overlapping. It was a clear limitation to this study that the subdivision of women into six age groups, resulted in rather small groups and with it very large CIs, thereby decreasing the chance of detecting a potential significant difference. Yet, the results of this comparison were in line with the previous finding that the pregnancy rate of women aged above 40 years was significantly higher in GKT compared to the national average, whereas the pregnancy rate in women younger than 40 years was corresponding. Hence, it appears that women of advancing age benefit the most from the KISS diet. However, the pregnancy rate was considerably higher than expected in women aged 40 years or above, and the sample sizes were relatively small. Therefore, it may be questioned if patients included in this study constituted a representative sample of the population.

It is well-known that insulin sensitivity decreases with advancing age, since several studies have demonstrated impaired glucose metabolism in old subjects, often above 60 years of age, compared to young subjects (27,103-105). DeFronzo (103) investigated the metabolism of glucose in 84 healthy subjects, divided into three age groups; a young group aged 21-29 years, a middle-aged group aged 30-49 years, and an old group aged 50-74 years. By means of the hyperinsulinemic-euglycaemic clamp technique, he discovered that the amount of metabolized glucose was significantly lower in both the middle-aged and old group compared to the young group. These results suggest that the age-related IR is already

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initiated in the third or fourth decade of life. Like in the hypothesis of this study, it may be assumed that women with the highest degrees of IR are most likely to experience improvements in their insulin sensitivity after an intervention, which aims at decreasing the IR. Therefore, the association between increasing age and IR may explain why women of advancing age benefit more from the KISS diet than younger women.

5.4 Comparison of spontaneous pregnancy rates

The spontaneous pregnancy rate of women receiving treatment with partner's semen in this study was 33.3 %. A multicenter study by Steeg et al. (106) included 3021 subfertile couples who were referred for an assessment of their infertility. These authors reported that 18 % achieved a spontaneous ongoing pregnancy within 12 months. Thus, it seems that the chance of conceiving spontaneously in GKT is superior to the one found by Steeg et al. (106). Another study by Keulers and colleagues (107) observed that the chance of conceiving spontaneously within a 12-month period in subfertile couples was 28.3 %, which is rather similar to the result of this study. However, in the studies by both Steeg et al. (106) and Keulers et al. (107) couples were only included if the woman had a regular menstrual cycle. By inclusion of patients with an irregular cycle, the proportion of women conceiving spontaneously would probably be smaller than reported by these studies. Furthermore, the time frame in which spontaneous pregnancy could occur was confined to 2-4 months in GKT. There is reason to believe that an equivalent time frame of 12 months would have increased the rate of women conceiving spontaneously. The potentially higher rate of spontaneous pregnancies in GKT may indicate that the KISS diet improves fertility. Again, a proper

control group was lacking, since factors such as advancing age, longer duration of infertility, and under- and overweight among others decrease the chance of achieving a spontaneous pregnancy (108). If the groups were different on these variables, a comparison may not be entitled.

5.5 The effect of diet composition on insulin sensitivity

Studies have tried to clarify the effect of macronutrient composition on clinical and biochemical features. Mornan et al. (109) randomized 28 overweight PCOS women to follow either a high protein (HP) diet (30 % protein, 40 % carbohydrates, 30 % fat) or a low protein (LP) diet (15 % protein, 55 % carbohydrate, 30 % fat). The calorie intake was restricted for the first 12 weeks, and weight maintenance was pursued in the four succeeding weeks. After completion of the 16 weeks' intervention, a significant weight loss was evident in both groups. Significant improvements in insulin sensitivity and regularity of menstrual cycles were also demonstrated, but these observations were not statistically significant between the diets. This suggests that diet composition does not influence insulin sensitivity and fertility. In another study by Stamets and colleagues (110), 26 obese PCOS patients received either a HP or LP diet with the exact same macronutrient distribution as described above. After four weeks, the subjects' weights had decreased and their insulin sensitivity was improved, evidenced by a reduced area under the curve for insulin. Further, there was a trend towards reduced fasting insulin (P=0.05) and glucose (P=0.05) levels after the diet interventions, but these results did not reach statistical significance, although it was faultily reported as significant by the authors. The improvements in insulin sensitivity did not differ between the groups, thus supporting the findings by Mornan et al (109). Similar results were found in diabetic patients in a study conducted by Parker and colleagues (111). They examined the influence of a HP (30 % protein, 40 % carbohydrate, 30 % fat) and a LP diet (15 % protein, 60 % carbohydrate and 25 % fat) in diabetic male and female patients. After 12 weeks on either the HP or LP diet, total and abdominal fat were reduced and insulin sensitivity, assessed by means of continuous low-dose insulin and glucose infusion, was improved in diabetic women compared to baseline. However, these findings were independent of the prescribed diet. All together, these studies indicate that neither subgroups of PCOS patients nor diabetic patients seem to benefit from a HP diet compared to a LP diet.

The lack of significance between diets in the three studies may result from inadequate statistical power because of rather small sample sizes. This was further deteriorated by relatively high dropout rates, which were approximately one third in two of the studies. It may also have influenced the results that the carbohydrate content exceeded the protein content in the high protein diets. Furthermore, no restrictions regarding the types of permitted carbohydrates were part of the diet interventions. Lower carbohydrate content and consumption of low/medium GI and GL foods only in the HP diets may enhance the possibility of detecting an effect of diet composition on IR and fertility if one exists.

In a study by Piatti et al. (112), 25 obese women were randomly assigned to a HP (45 % protein, 35 % carbohydrate, 20 % fat) or LP diet (20 % protein, 60 % carbohydrate, 20 % fat) for three weeks. Both diets were hypocaloric. In all women, normal blood glucose values in an OGTT were obtained. The insulin sensitivity was evaluated at baseline and at the end of the diet intervention by the hyperinsulinemic-euglycemic clamp technique. This test was only performed in eight subjects from each group. It was revealed that the glucose uptake and oxidation after the diet interventions were significantly higher in the HP group compared to the LP group, signifying improved insulin sensitivity in the HP group. Contrary to the above studies, these results suggest that a diet high in protein may alleviate IR. Consequently, it is possible that a HP diet may also cause improvements in the reproductive outcome.

The discrepancy between the above studies may be explained by the higher protein and lower carbohydrate contents employed by Piatti et al. Furthermore, HP diets may have varying impact on different subgroups.

5.6 A call for additional studies

The current research is conflicting, and it is questionable whether the study designs have been optimal for detecting a potential difference between different diet compositions. Larger, preferably multicenter studies using a very high protein content are required.

In this study, limitations were present as described in previous paragraphs, and the effect of the KISS diet on fertility outcome remains to be fully elucidated. In a more ideal study, infertile women should be randomized to either KISS diet or a control diet with a high carbohydrate content of e.g. 50-60 %. The duration of the diet intervention should be carefully selected, since detection of a potential effect could be missed if the study period is too short. On the other hand, compliance issues may arise if the study period is too long. The compliance of both groups should be regularly assessed, remedied by food diaries, dietetic or medical follow-up, or urine tests measuring concentrations of e.g. creatinine. The primary outcome measures could be live birth rates or pregnancy rates. Changes in hormonal levels, including androgen levels, and insulin sensitivity before and after diet intervention between the groups would also be of interest. In addition, parameters evaluating oocyte quality and endometrial receptivity could also be addressed, since it has been suggested that both are associated with insulin resistant infertility, cf. section 1.3.2 and 1.3.3.

5.7 Advantages of the KISS diet

Obviously, not all infertile women are eligible for treatment with KISS diet because severe male factor and tubal infertility as a main rule must be managed with IVF. But if future studies confirm a beneficial effect of KISS diet on infertility, maybe in certain subgroups, the diet should be propagated as an alternative or a supplement to the traditional fertility treatments because of its advantages.

Firstly, the utilization of hormones for follicle maturation and ovulation induction may be reduced. This means that fewer patients will experience adverse drug effects. Further, hormonal treatment increases the risk of multiple pregnancies (113). Probably as a result of the modest administration of hormones in GKT, the multiple pregnancy rates in women treated with partner's semen were 2.3 % in women below 40 years of age and 0 % in women 40 years of age or above. These rates are extremely low compared to rates reported by the NDFS. They reported that 10.3 % and 14.3 % of the pregnancies were multiple among women <40 years and \geq 40 years, respectively (96). Similar results are demonstrable for women inseminated with donor semen. It should be noted that the sample size for women older than 40 years of age was relatively small. This may have resulted in an incorrect representation of the distribution in GKT

Secondly, implementation of KISS diet as a standard option of fertility treatment would probably entail reduced costs for the public health care system. In a recent analysis the direct economic costs (comprising labour and materials) per IUI cycle were found to be 851€ or 6335 DKK (114). It is highly probable that expenses to labour and materials are lower for a diet intervention, but an economic evaluation such as a cost effectiveness analysis is necessary to precisely determine the difference in costs per successful outcome.

Thirdly, weight loss per se improves fertility outcome in overweight and obese infertile women (115-117) and may be recommended as first-line treatment for infertility. However, weight loss is not an option for lean women. KISS diet is applicable for all patients regardless of weight as it does not focus on calorie restrictions and weight loss.

6. Conclusion

The aim of this study was to investigate the influence of the KISS diet on the reproductive outcome of infertile women. Baseline Cpeptide levels were found to be similar in patients who became pregnant and patients who did not. This was in contrast to the hypothesis, stating that women with high degrees of IR, i.e. high baseline C-peptide levels, would be better responders of the KISS diet. An explanation for this deviation may be that a single baseline measurement of C-peptide is not an ideal surrogate of insulin sensitivity.

The pregnancy rate for women inseminated with donor semen under the age of 40 years was comparable to the national average. However, the pregnancy rate for women aged 40 years or older treated with donor insemination was higher than the national average. Compared to live birth rates from the United Kingdom, the outcome was better in GKT for women aged 40-42 years. Taken together, these results indicate that the diet improves the fertility treatment outcome in women of advancing age, who normally have a high failure rate.

Regarding spontaneous pregnancy rates, it appeared that women in GKT more often achieved an ongoing spontaneous pregnancy than reported by other studies (106,107). This may suggest that the reproductive outcome of infertile women is improved by the KISS diet.

In the absence of a proper control group and more suitable variables, the conclusions drawn from this study are mainly indicia. Therefore, additional well-designed and large studies are required in order to clarify the effect of the KISS diet on the reproductive outcome of infertile patients.

7. Acknowledgements

I wish to thank Bjarne Stigsby for putting the data at my disposal and for his valuable advice during the making of this thesis. I also gratefully acknowledge Linda Pilgaard for her helpful guidance and support.

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