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REVIEW ARTICLE

The association of physiological cortisol and IVF treatment outcomes: a systematic review

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Abstract
Purpose A systematic review was conducted to (1) collate and synthesise the available evidence for the role of cortisol in relation to IVF treatment outcomes; (2) to establish the strength of an association between cortisol and IVF; and (3) to assess the overall quality of the studies and guide future research in this area.
Methods Seven electronic databases, including the reference lists of published papers, were searched. Inclusion criteria qualified any prospective/observational cohort study that reported original data. Quality assessment of eligible studies was conducted using the STROBE statement, which was used to assess the risk of bias and the quality of observational studies included in this review.
Result(s) A total of eight studies reported a significant association between cortisol and IVF outcomes. Three studies found that higher cortisol may be associated with more favourable IVF outcomes, whereas five studies found that lower cortisol levels may be conducive to IVF success. Eleven of all studies included in this review were regarded as low quality publications.
Conclusion(s) Study findings were that the evidence for the role of cortisol in relation to IVF outcomes is currently mixed. Future researchers are encouraged to consider the methodological limitations highlighted in this review and to utilise more robust assessment methods when examining the influence that chronic, rather than acute, stress may have on IVF outcomes.

Keywords Cortisol · Hypothalamus pituitary adrenal (HPA) axis · Infertility · In Vitro Fertilisation (IVF) · Stress

Introduction

An area that has received growing interest in recent years is the potential role that psychological stress may play in determining in vitro fertilisation (IVF) treatment outcomes. At least anecdotally, many infertile women and health care professionals alike believe that the experience of stress can play an important role in the difficulties that infertile patients face, and thus may be a contributing factor in determining the eventual outcome of IVF [1]. Whilst research in this area has been mixed, two recent systematic reviews suggest that self-reported psychological stress may well play a role in determining a patient's IVF outcome. However, both reviews highlight the need for further research which attempts to elucidate the psychobiological pathways which may mediate a putative stress and IVF association [2, 3]. Despite the apparent research interest in the role of stress and IVF, no systematic review has been conducted to date on the role of cortisol, a biological

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concomitant of psychological stress, and the role this may play in determining IVF treatment outcomes.

Indeed, one psychobiological pathway by which stress is commonly thought to impact upon reproductive functioning is through activation of the hypothalamus pituitary adrenal (HPA) axis. This system is considered central to the human stress response and, upon activation, results in the secretion of the stress hormone cortisol, which is generally released in higher doses under stressful conditions [4]. Cortisol is a glucocorticoid hormone which plays an important role in numerous processes including metabolism, blood pressure, and immune response regulation, and thus has proved a reliable biological correlate of many adverse health outcomes [5]. In the context of IVF, a growing body of evidence suggests that stress may exert its deleterious effects on IVF treatment outcomes through activation of the HPA axis [6–11].This hypothesis is plausible because both physical and emotional stress can cause alterations to the endocrine axis which may, in turn, affect the reproductive system through immunosuppression [12]. However, although a relationship between the HPA axis and reproductive success is possible, the evidence for a cortisol and IVF association appears inconclusive, with a number of studies reporting an association [7, 8, 11, 13–15] and others reporting no association between cortisol levels and IVF treatment outcomes [9, 10, 16–18]. In addition, there also exists ambiguity within the literature regarding the directionality of a potential cortisol/IVF relationship. That is, it remains unclear as to whether higher or lower cortisol levels are detrimental or conducive to optimal reproductive functioning. Despite the clinical importance of the research and the efforts made to better understand the stress/IVF relationship, no systematic review to date has collated and synthesised the available evidence for the role of cortisol in relation to IVF treatment. Therefore, in order to address the uncertainty within this body of work, we conducted a systematic review of 25 years of research that has reported data on levels of cortisol as measured in blood, urine, saliva and follicular fluid, and a range of IVF treatment outcomes including clinical pregnancy, oocyte number, oocyte fertilisation, oocyte cleavage, and miscarriage rates. We feel that a systematic review is timely and warranted in this area because, whilst previous reviews are available on the role of negative effects/stress and IVF, no systematic review to date has been conducted on the role of cortisol in relation to IVF. This is surprising given the role of cortisol as a biological concomitant of psychological stress. The primary aims of this review, therefore, are; (1) to collate and synthesise the available evidence for the role of cortisol in relation to IVF treatment outcomes, (2) to establish the strength of an association between cortisol and IVF outcomes, and (3) to assess the overall quality of the studies within this area and highlight the methodological priorities and associated design implications to help guide future research.

Methods

Systematic search

Search methods, criteria for inclusion, and outcomes were specified in advance and documented in the protocol, which was registered with PROSPERO on 7th January 2013 (PROSPERO registration number: CRD42013003566). No limitations were placed on language or publication date. Commentaries, letters and conference abstracts were included. A systematic search of MEDLINE, EMBASE, PSYCHInfo, Psycharticles, Web of Knowledge, PubMed, and CINAHL was conducted by two reviewers. The search was last conducted on 10th August 2013. The following search terms were used and adjusted for each database as necessary: (cortisol) or (hydrocortisone) or (hypothalamus pituitary adrenal axis) or (hypothalamus pituitary adrenal gonadal axis), and (IVF treatment) or (in vitro fertili*ation) or (infertility) or (assisted reproduction). Limits placed on the search were full text and humans. A comprehensive examination of the reference sections of all identified publications was also conducted to identify other relevant publications. All identified citations were transferred to EndNote (Thomson Reuters, San Francisco, CA, USA).

Study selection

Inclusion criteria were any prospective/observational cohort study that reported original data on the association between cortisol and treatment outcomes in relation to IVF including intracytoplasmic sperm injection (ICSI) or frozen embryo transfer (FER) cycles. The IVF outcome variables included number of oocytes retrieved, oocyte cleavage, oocyte fertilisation rates, miscarriage rates, and clinical pregnancy. Two independent reviewers (A.M. and N.A) screened the retrieved titles and abstracts using the inclusion and exclusion criteria. Articles were included for full text review if the reviewers considered the study to be appropriate on the basis of the title/abstract screening. Disagreements regarding the inclusion of a paper were resolved by consensus or by a third party (KV). Reasons for exclusion included (1) not reporting absolute cortisol levels (e.g. reporting the cortisol/cortisone ratio only), (2) investigating cortisol levels in infertile populations only in relation to fecundity or menstrual cycle phase, (3) not reporting on associations between cortisol and outcomes of IVF treatment, (4) clinical trials investigating interventions likely to perturb cortisol levels, and (5) no full text available or provided by authors upon request.

Data extraction

Data extraction was conducted independently by two reviewers (A.M and N.A) using a data extraction form which was designed specifically for this review. In the case of missing or inconsistent data, authors were contacted to provide further information. The following study characteristics were extracted from the included studies: study design, time period, population, inclusion and exclusion criteria, treatment outcomes measured, confounding factors (e.g. smoking status, BMI, glucose, caffeine, menstrual cycle phase), number of cortisol measures, method of cortisol collection, and fertility diagnosis.

Data synthesis

Owing to considerable heterogeneity in study design and variations in how data were presented in each reviewed study, it was not possible to use a meta-analytic approach to review studies included in this review. Several authors were contacted to provide further information but were unable to do so. Therefore, a descriptive account of all studies was prepared in order to summarise, synthesise, and evaluate all studies included in this review.

Quality assessment

The STROBE (strengthening the reporting of observational studies in epidemiology) statement, which is a robust and widely used directive employed to guide the reporting of observational studies [19], was used by two authors (A.M and N.A) to assess the quality of studies included in this review. The resulting quality and risk of bias assessment tool developed for this review comprised of 8 core domains: (1) *Study design and setting*: studies were awarded a point for this criterion if key elements of study design were described including descriptions of setting, locations, relevant dates, periods of recruitment, follow-up periods, and data collection methods used; (2) *Descriptions of inclusion and exclusion criteria*: studies were awarded a point for this criterion if participant eligibility criteria were clearly described including the sources and methods used to select participants; (3) *Definition of variables and measurements*: studies were awarded a point for this criterion if study outcomes, exposures, predictors, and potential confounders were clearly described; (4) *Confounding variables*: studies were awarded a point for this criterion if efforts were made to control for confounders and potential sources of bias in each study; (5) *Sample size*: studies were awarded a point for this criterion if an adequate sample size was used and appropriate statistical measures were described. The statistical methods used in each study were also assessed and one mark was awarded for each of the following criteria (6) *Confounding factors*: a description of statistical analyses used to control for confounding and potential sources of bias; (7) *Missing data*: a description of how missing data was addressed; (8) *Outcome estimates and measures of variability*: a study was awarded one point for this criterion if outcome estimates and measures of variability were provided by the authors of each study. This gave a total score of 8 points for each study. Those scoring between 0 and 3 points were considered low quality, studies scoring 4–6 points were considered to be of satisfactory quality, and those scoring between 7 and 8 points were considered to be high quality studies. Table 1 below summarises the results of the quality analysis for each study.

Results

Description of studies

A flow chart of study selection and inclusion of eligible studies is summarised in Fig. 1. Electronic and manual searches yielded 770 potential papers. Once duplicates had been removed papers were screened (A.M and N.A) for inclusion, which yielded a total of 22 papers eligible for our review. Several authors were contacted to provide further information but were unable to provide additional data. Six papers were excluded from the review with reasons. Sixteen papers met the inclusion criteria and were included in the review; their characteristics are shown in Table 2 below.

STROBE quality assessment

STROBE ratings indicated low study quality for eleven of the sixteen studies which assessed cortisol and IVF treatment outcomes. Two studies were considered to be of satisfactory quality, with a further three studies considered high quality papers. See Table 1 for a summary of quality assessment.

Sample characteristics

The included studies sampled 1,647 female patients in eight countries. The mean age of the participants was 32.9 years. Sample sizes of the included studies ranged from 14 to 387 participants, with many of the published studies lacking statistical power or failing to report power calculations (>0.80 with an alpha of 0.05) [6, 7, 13, 14, 20–22]. Nine of the reviewed studies included patients with a range of infertility diagnoses (e.g. male factor/female factor/idiopathic/mechanical infertility/minimal endometriosis/luteal phase insufficiency, etc.). Two studies included

Table 1 Quality and risk of bias assessment using the STROBE guidelines

Author	Study design and setting	Inclusion and exclusion Criteria	Definition of variables and measurements	Confounding variables (smoking, BMI, glucose, caffeine, time of sampling, menstrual cycle)	Sample size	Statistical methods		
						Confounding factors	Missing data	Outcome estimate and measures of variability (CIs)
Fatah et al. [6]	x	x	x	x	x	x	x	x
Demyttenaere et al. [7]	•	x	x	x	x	x	x	x
Andersen and Hornnes [13]	x	x	x	•	x	x	x	x
Bider et al. [20]	•	•	x	x	x	x	x	x
Milad et al. [21]	•	•	•	x	x	x	x	x
Anderson et al. [22]	•	•	•	x	x	x	x	x
Micheal et al. [8]	•	x	•	x	x	x	x	x
Csemiczky et al. [9]	x	•	•	x	x	x	x	x
Keay et al. [14]	x	•	•	x	x	x	x	x
Lewicka et al [10]	•	x	x	x	•	x	x	•
Lovely et al. [16]	•	•	•	•	•	x	x	x
Thurston et al. [15]	•	x	•	x	•	x	x	x
Smeenk et al. [17]	•	x	•	x	•	x	•	x
An et al. [11]	•	•	•	•	•	•	•	x
Nouri et al. [18]	•	•	•	•	•	x	•	•
An et al. [23]	•	•	•	•	•	•	•	x

•, study considered to be of satisfactory quality in this area; x, study considered not to have met standards of satisfactory quality in this area

only patients with tubal factor infertility, and five studies failed to report any infertility diagnosis at all.

Cortisol collection methods

Seven studies relied on a single method of cortisol collection only. That is, three studies used blood sampling; two studies used FF, and a further two studies used urine collection methods. The remaining nine studies used a combination of collection methods, i.e. seven studies used blood and FF sampling combined, whilst two studies utilised blood and saliva collection methods combined.

Stage of treatment

Nine of the included studies relied on measures of cortisol taken at one stage of the IVF treatment cycle. Two studies measured cortisol during the down regulation phase only [16, 18]; six studies measured cortisol during the oocyte retrieval phase only [8, 10, 13, 15, 20, 22], whilst one study measured cortisol solely during embryo transfer [21]. Seven studies assessed cortisol over multiple stages of the treatment cycle [6, 7, 9, 11, 14, 17, 23].

Cortisol and clinical pregnancy

Twelve studies in total assessed the association of cortisol in relation to the establishment of pregnancy. Three studies reported that elevated cortisol levels were observed in patients obtaining a clinical pregnancy [13–15]. However, in contrast, four studies reported that higher cortisol levels were observed in those patients failing to conceive through IVF [7, 8, 11, 23]. Five studies reported no significant differences in cortisol concentrations between the conception and non-conception groups [9, 10, 16–18].

Cortisol and IVF outcomes (oocyte number, fertilisation rates, cleavage, and miscarriage rates)

Three of the included studies assessed the relationship between cortisol and the number of oocytes retrieved. One study found that lower cortisol values were related to a greater number of oocytes [7]. However, two studies found no association between cortisol levels and oocyte number [13, 18]. Four studies examined the role of cortisol and oocyte fertilisation. Two studies found that higher cortisol was associated with oocytes that did not fertilise [6, 7]. However, one study

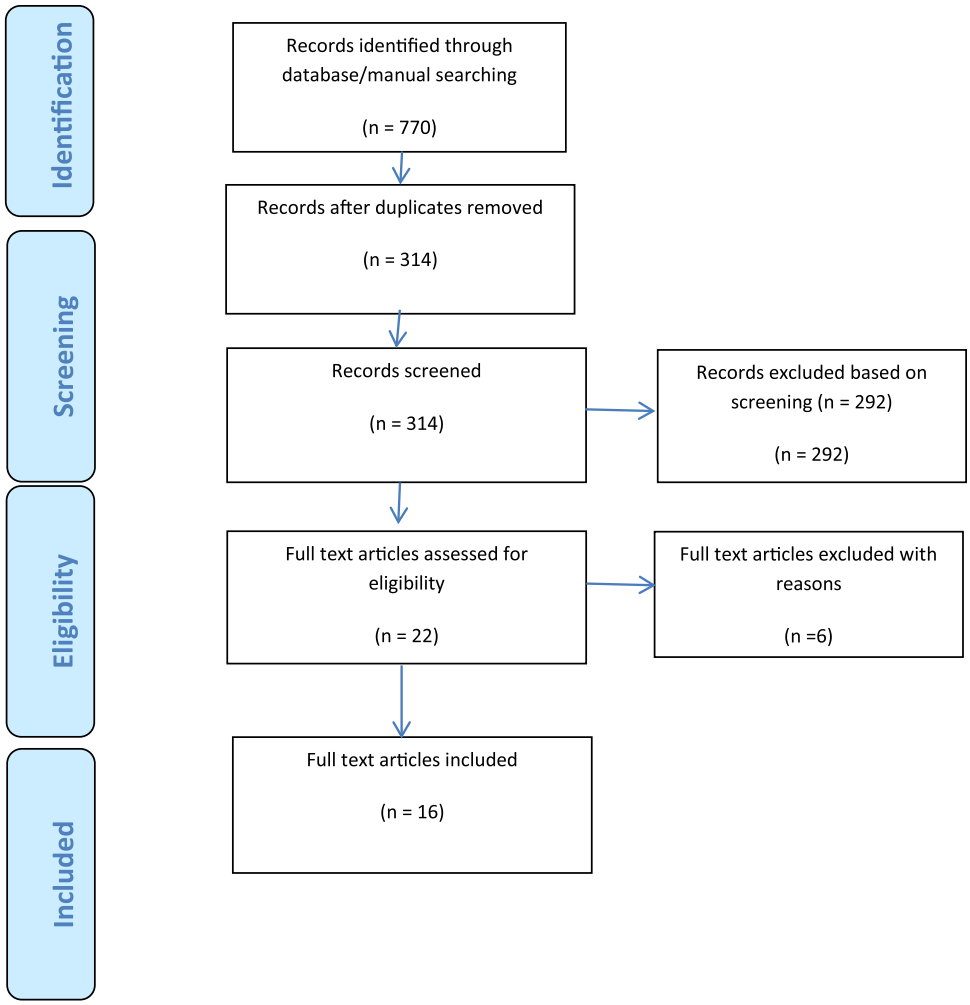

Fig. 1 PRISMA Decision Flow chart for Identified Studies

found that higher cortisol was associated with oocytes that did fertilise [14]. In contrast, one study found no association between cortisol levels and oocyte fertilisation rates [20]. Three studies examined cortisol and oocyte cleavage. One study found that lower cortisol levels were associated with oocytes that cleaved [7]. However, two studies found no association between cortisol levels and oocyte cleavage potential [6, 22]. Two studies assessed oocyte maturity. One study found that higher cortisol levels were associated with follicles containing mature oocytes [6]. This is in contrast to one study that found no association between cortisol levels and oocyte maturity [13]. Finally, one study examined cortisol and miscarriage rates and in relation to IVF [21]; its results suggested that that there was no significant association between cortisol and miscarriage rates.

Discussion

This is the first study to systematically review the available literature on the relationship between cortisol and a range of IVF treatment outcomes. We employed robust methods to assess the quality and scientific rigour of over two decades of research conducted in this area. Overall, our findings suggest that 69 % of studies examining the role of cortisol in relation to IVF outcomes from 1989 to 2013 were considered to be of low scientific quality. Whilst our findings suggest that the available cortisol/IVF data is disappointingly poor, we feel that the lack of quality evident in this area emphasises the need for a robust systematic review which highlights the salient methodological issues and offers direction to improve and guide future studies in this area.

Clinical pregnancy was the most frequently reported IVF outcome, with four studies reporting that lower cortisol was associated with the establishment of clinical pregnancy [7, 8, 11, 23] and three studies reporting an association between higher cortisol levels and pregnancy rate [13–15]. Of notable interest is that the aforementioned studies report data derived from follicular fluid measures of cortisol. Whereas four of the studies that report no significant differences in cortisol between pregnant and non-pregnant groups report data derived from blood, saliva, or

Table 2 Study characteristics of studies included in the review

First author	Participants	Inclusion criteria/ exclusion criteria	Treatment outcome variables	Sampling method (saliva, blood, follicular fluid, urine)	Number of measures	Stage of treatment/time of day	Confounding variables (smoking, BMI, glucose, caffeine, time of sampling, menstrual cycle)	Fertility diagnosis	Main outcomes
Cortisol variables									
Fateh et al. [6]	67 patients (age not stated)	*Inclusion* (1) Not obese (2) Free of renal (3) Hepatic and endocrine diseases (4) History of normal menstrual cycle *Exclusion* Not stated	Oocyte fertilisability Oocyte cleavage Oocyte maturity	Follicular fluid Blood sample	1 × follicular fluid 1 × blood sample	During the follicular phase, oocyte retrieval and embryo transfer phase of treatment. Time of day not stated	Menstrual cycle	Mechanical causes of infertility	Higher cortisol was associated with oocytes that did not fertilise. There was no association between cortisol levels and embryo cleavage. Higher cortisol was associated with follicles containing mature occytes.
Demyttenaere et al. [7]	40 females (mean age 32.4 years)	*Inclusion* Not stated *Exclusion* Not stated	Oocyte number Fertilisation rate Oocyte cleavage Establishment of pregnancy	Blood sampling	7 × blood sampling	(1) Pre-treatment during follicular phase of menstruation (2) Morning of oocyte retrieval (3) In the afternoon of embryo transfer (–90, –60, –30 min before the procedures and +30, +60, +90, +120 min post-procedure	Not stated	Male subfertility (n = 11), mechanical infertility (n = 10), both combined (n = 6), unexplained infertility, minimal endometriosis and luteal phase insufficiency (n = 13)	Early follicular phase cortisol and mid-follicular phase cortisol concentrations were negatively correlated with clinical pregnancy. Lower cortisol was associated with a greater number of oocytes and improved fertilisation rates and cleavage.

Table 2 continued

First author	Participants	Inclusion criteria/ exclusion criteria	Treatment outcome variables	Sampling method (saliva, blood, follicular fluid, urine)	Number of measures	Stage of treatment/time of day	Confounding variables (smoking, BMI, glucose, caffeine, time of sampling, menstrual cycle)	Fertility diagnosis	Main outcomes
Andersen and Hornnes [13]	14 females (7 pregnant, mean age 32.5; 7 non pregnant, mean age 31.1)	*Inclusion* (1) Tubal factor causes of infertility (2) Normal ovulation (3) Normal semen quality according to WHO criteria for male partner *Exclusion* Not stated	Oocyte number Fertilisation rate Oocyte maturation Quality of ovulation Induction Quality of luteal phase Establishment of pregnancy	Blood sampling Follicular fluid	1 × follicular fluid 1 × blood sample	Oocyte recovery/ time of day not stated	Not stated	Tubal factor infertility	The free and total cortisol concentrations in follicular fluid were associated with oocyte cleavage and establishing pregnancies. In FF from pregnant women free cortisol was significantly higher in pregnancy-associated follicles. No associations were found between cortisol and oocyte number.
Bider et al. [20]	Low responder group (n = 20, mean age = 38.2 years) Good responder group (n = 15, mean age = 32.1 years)	*Inclusion* Not stated *Exclusion* Not stated	Oocyte fertilization	Follicular fluid Blood sampling	1 × follicular fluid 1 × blood sample	Oocyte retrieval/ time of day not stated	Not stated	Tubal unexplained fertility	Total cortisol concentrations were not associated with oocyte fertilisation
Milad et al. [21]	40 participants (31.3/35.2 years)	*Inclusion* Patients undergoing controlled ovarian stimulation *Exclusion* Not stated	Miscarriage rate	Saliva Blood sampling	3 × saliva 3 × blood samples	13, 20, and 27 days post-embryo transfer early in pregnancy Time of day not stated	Not stated	Male factor Tubal factor endometriosis Ovulation dysfunction Uterine abnormality unexplained	Cortisol measures were not related to miscarriage rate

Table 2 continued

First author	Participants	Inclusion criteria/ exclusion criteria	Treatment outcome variables	Sampling method (saliva, blood, follicular fluid, urine)	Number of measures	Stage of treatment/time of day	Confounding variables (smoking, BMI, glucose, caffeine, time of sampling, menstrual cycle)	Fertility diagnosis	Main outcomes
Anderson et al. [22]	Eleven women in their natural cycle having an endogenous mid cycle surge of gonadotropins to induce ovulation (mean age 34 years) Seven women in their natural cycle where HCG was used to induce ovulation (mean age 32 years) Six women just before they received HCG (mean age 27 years)	*Inclusion* Not stated *Exclusion* Not stated	Clinical pregnancy Occyte cleavage/ implantation	Blood sampling Follicular fluid (FF) samples representing four different types of pre ovulatory FF were included: (1) 11 femalesin their natural cycle having an endogenous mid cycle surge of gonadotropins to induce ovulation (2) Seven women in their natural cycle where HCG was used to induce ovulation (3) Six women just before they received HCG (mean age 27 years)	1 × blood sample 1 × follicular fluid	During oocyte recovery	Not stated	Not stated	No association was found between cortisol levels and oocyte cleavage No significant associations were found between cortisol levels and clinical pregnancy outcome
Micheal et al. [8]	23 females (age not stated)	*Inclusion* Not stated *Exclusion* Not stated	Establishment of pregnancy	Blood sampling Follicular fluid	1 × follicular fluid 1 × blood samples	Oocyte recovery/ time of day not stated	Not stated	Not stated	Cortisol concentrations were significantly lower in the group obtaining a pregnancy compared to the non-conception cycles.

Table 2 continued

First author	Participants	Inclusion criteria/ exclusion criteria	Treatment outcome variables	Sampling method (saliva, blood, follicular fluid, urine)	Number of measures	Stage of treatment/time of day	Confounding variables (smoking, BMI, glucose, caffeine, time of sampling, menstrual cycle)	Fertility diagnosis	Main outcomes
Csemiczky et al. [9]	22 females (Infertile, 33.4 years; Fertile, 33.1 years)	*Inclusion* All women were regularly menstruating and underwent an investigation for infertility including basal body temperature, characterization of the menstrual cycle by repeated serum levels of follicle stimulating hormone, estradiol, and progesterone in the luteal phase *Exclusion* Not stated	Establishment of pregnancy	Blood sampling	16 × blood samples	Throughout natural cycle from day 3,10–15, and 19–26 Time of day not stated	BMI	Tubal infertility	Serum measures of cortisol revealed no significant differences in concentrations between the conceiving group and non-conceiving group.
Keay al. [14]	42 females (<40 years)	*Inclusion* Women <40 years old with unexplained infertility, tubal infertility, or minor endometriosis were included in the study *Exclusion* Not stated	Establishment of pregnancy fertilisation rates	Follicular fluid	1 × follicular fluid	From day 9 of the unstimulated cycle to oocyte retrieval Time of day not stated	Not stated	Unexplained fertility, Tubal infertility, or Minor endometriosis	Clinical pregnancy was associated with higher cortisol concentrations. Significantly higher cortisol was associated with fertilisation potential.
Lewicka et al. [10]	387 females (22–46 years)	*Inclusion* Not stated *Exclusion* Not stated	Establishment of pregnancy	Follicular fluid Blood sampling	1 × follicular fluid 1 × blood samples	Oocyte retrieval	Not stated	Not stated	There was no significant difference in follicular fluid or serum cortisol concentrations between pregnant women and the non-pregnant women.

Table 2 continued

First author	Participants	Inclusion criteria/ exclusion criteria	Treatment outcome variables	Sampling method (saliva, blood, follicular fluid, urine)	Number of measures	Stage of treatment/time of day	Confounding variables (smoking, BMI, glucose, caffeine, time of sampling, menstrual cycle)	Fertility diagnosis	Main outcomes
Lovely et al. [16]	42 females (31.2 years)	*Inclusion* Ovulatory dysfunction Tubal disease Male factor Unexplained infertility *Exclusion* Not stated	Establishment of pregnancy	Urine	24 h	Participants collected a 24 h urine specimen on the day after gonadotrophin administration	Not stated	Ovulatory dysfunction (n = 12) Tubal disease (n = 13) Male factor (n = 18) Unexplained infertility (7 patients)	The women who subsequently became pregnant had higher urinary cortisol levels compared with the non-pregnant group. However, this difference was not significant.
Thurston et al. [15]	132 females (23–43 years)	*Inclusion* Not stated *Exclusion* Not stated	Establishment of pregnancy	Follicular fluid	1 × follicular fluid	Oocyte recovery/ time of day not stated	Not stated	Not stated	Concentrations of cortisol were associated significantly with the probability of conception. Cortisol concentrations were significantly elevated in FF obtained from conception cycles relative to samples from non-conception cycles.
Smeenk et al. [17]	168 females (34.3 years)	*Inclusion* Not stated *Exclusion* Not stated	Establishment of pregnancy	Urine	3 × urine measures taken pre-treatment, before oocyte retrieval, and before embryo transfer	Day 10 and 20 of the premedication cycle and before embryo transfer	Not stated	Not stated	No significant differences in cortisol were found between those conceiving and the non-conception groups.

Table 2 continued

First author	Participants	Inclusion criteria/ exclusion criteria	Treatment outcome variables	Sampling method (saliva, blood, follicular fluid, urine)	Number of measures	Stage of treatment/time of day	Confounding variables (smoking, BMI, glucose, caffeine, time of sampling, menstrual cycle)	Fertility diagnosis	Main outcomes
An et al. [11]	264 (pregnant group, 33.1 years; n on-pregnant group, 33.4 years)	*Inclusion* Only women with regular menstrual cycles and using no hormonal contraceptives *Exclusion* Smokers, acute or chronic hormonal dysregulations, ovarian endometriosis, any psychosomatic or psychiatric diseases	Establishment of pregnancy	Blood sampling Follicular fluid	1 × blood sample 1 × follicular fluid	During the luteal phase before treatment and on day of oocyte retrieval (8–9 a.m.)	Age Smokers Ovarian endometriosis Psychiatric disease	Mild male/ pregnant group (18) Severe male/ pregnant (group (29) Unexplained/ pregnant group (14) Female/non-pregnant group (63) Mild male/non-pregnant group (28) Severe male/non-pregnant group (52) Unexplained/non-pregnant group (29)	Significantly higher levels of cortisol were found in the FF of patients who failed to conceive through IVF.
Nouri et al. [18]	83 females (29.0 years)	*Inclusion* A diagnosis of infertility based on male, female, or idiopathic factors; Willingness to participate in the study; Signed informed consent; First IVF cycle using an antagonist protocol *Exclusion* Not stated	Number of oocytes Establishment of pregnancy	Saliva Blood sampling	6 × saliva samples	Patients were asked to collect saliva samples within 30 min of awakening and before going to bed on day 1, 2, and 3 of the IVF cycle	Not stated	Male factor Female factor Idiopathic	No significant associations were found between cortisol and the establishment of a clinical pregnancy (data not shown). No association was reported between cortisol and oocyte number.

Table 2 continued

First author	Participants	Inclusion criteria/ exclusion criteria	Treatment outcome variables	Sampling method (saliva, blood, follicular fluid, urine)	Number of measures	Stage of treatment/time of day	Confounding variables (smoking, BMI, glucose, caffeine, time of sampling, menstrual cycle)	Fertility diagnosis	Main outcomes
An et al. [23]	264 females (33.1 years) Non-pregnant group (33.4 year)	*Inclusion* Only women with regular menstrual cycles and using no hormonal contraceptives *Exclusions* Smokers, acute or chronic hormonal dysregulations, ovarian endometriosis, any psychosomatic or psychiatric diseases	Clinical pregnancy	Blood sampling	4 × blood samples	Blood samples were collected at four time points throughout the treatment cycle (1) Pretreatment (2) Day of occyte retrieval (3) The day of pregnancy test (4) For those patients conceiving, at 5–8 weeks of gestation	Age Smokers Ovarian endometriosis Psychiatric disease	Mild male/ pregnant group (18) Severe male/ pregnant group (29) Unexplained/ pregnant group (14) Female/non-pregnant group (63) Mild male/non pregnant group (28) Severe male/non-pregnant group (52) Unexplained/non-pregnant group (29)	Lower levels of cortisol at oocyte retrieval and pregnancy test were significantly associated with the establishment of clinical pregnancy.

urine sampling [9, 16–18]. Whilst it is unlikely that sampling method may account for all of the observed heterogeneity between studies, it is important to acknowledge that concentrations of free biologically active cortisol derived from follicular fluid have been reported to be 10 times higher than those found in serum [13]. Furthermore, studies also varied in the reporting of free biologically active cortisol and total cortisol levels. However, salivary cortisol predominantly reflects the free biologically active fraction of cortisol, and whilst salivary cortisol agrees very well with the amount of free cortisol in blood, it often fails to show high correlations with total cortisol levels [24–26]. Indeed, absolute levels of cortisol are considered to be lower in saliva compared to blood due to a relative abundance of the cortisol-metabolizing enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD-2) converting active cortisol into inactive cortisone [27, 28]. These studies underline the importance of strictly distinguishing between total cortisol secretion and the levels of bioavailable free cortisol, as can be measured in saliva. Indeed, whilst these factors may not compromise comparability within studies, it certainly restricts comparability between studies, particularly when different methods of cortisol sampling have been used. Therefore, we recommend that both inactive and biologically active forms of cortisol be reported where possible. A further five studies conducted in this area failed to find any association between cortisol and clinical pregnancy outcome. A number of methodological limitations highlighted in our review which may account for further variance observed within the literature will now be discussed.

Stage of treatment and time points of assessment

The reviewed studies differed in their assessment of downregulation, oocyte retrieval, and embryo transfer stages of the treatment process. However, IVF treatment is inherently heterogeneous, and stress and the concomitant cortisol levels are likely to differ at different stages of the treatment process [2, 3]. Therefore, administering only single measures during one stage of a somewhat longitudinal treatment process is unlikely to optimally capture and reflect the role of the HPA axis. Indeed, salivary collection methods undoubtedly provide the most efficient means of ambulatory monitoring compared to urine, blood, and follicular fluid methods, particularly when attempting to assess cortisol levels throughout multiple stages of the IVF cycle. The typical methodologies used in other areas of cortisol research range from a 'minimal protocol', in which three samples are collected per person at different time points throughout a single day, to a 'high intensity' protocol which may, for example, involve six samples on a single day across multiple time points. We recommend that future researchers aspire to use 'high intensity' protocols which are considered to be more rigorous and may be more suited to the context of IVF. However, whilst 'high intensity' protocols are considered the gold standard, we acknowledge that cortisol sampling can be costly and recommend that the financial implications of multiple testing protocols be factored into the design of studies, particularly when dealing with larger samples.

Time of day

Cortisol is understood to follow a diurnal circadian rhythm in which levels are characterised by a surge in cortisol that occurs 30–45 min after awakening, the so called cortisol awakening response (CAR), and decrease gradually throughout the day. However, several of the studies included in this review failed to detail the time of day that cortisol sampling was administered. It is particularly important that future researchers ensure that cortisol sampling procedures are standardised within studies so that comparisons between groups are not confounded by time of day.

Explaining the heterogeneity: the role of extraneous variables

Our review highlights a number of additional covariates which may account for the mixed findings found within the literature. Our quality assessment suggests that only 31 % of studies conducted in this area were considered to have satisfactorily accounted for the many known covariates understood to influence HPA axis activity. On the whole studies were weak at controlling for these factors, and we hypothesise that failure to control extraneous variables within studies may contaminate the reported findings.

Nicotine is one extraneous variable and a potent stimulator of the HPA axis largely overlooked by studies in our review [for reviews see 29–32]. Failing to account for smoking status may therefore falsely exaggerate resulting cortisol levels and may potentially account for some of the inter- and intra-individual variation observed in the studies in this review. It has been shown that caffeine intake prior to sampling may also superficially increase plasma and saliva cortisol levels [33]. In addition, menstrual cycle phase is understood to influence HPA axis activity and thus may account for some degree of intra-individual variation also in that women in the luteal phase show significantly higher cortisol responses compared to follicular phase women [34]. In addition, body mass index [35] is an extraneous variable which should be routinely reported in relation to cortisol data but was overlooked by studies in our review.

What is high? What is low?

An important consideration largely overlooked within the literature and highlighted in our review is what values are used to dichotomise high and low cortisol between studies. For example, Andersen and Hornnes [13] reported that higher cortisol values (mean 234.0 nmol/l) were associated with clinical pregnancy compared to lower values. These values are comparable to the study conducted by Keay et al. [14] which also suggested that higher values of cortisol (mean 299 nmol/l) were associated with clinical pregnancy outcome. However, in contrast to these findings, Micheal et al. [8] concluded that lower values (mean 304.0 nmol/l) were related to establishment of clinical pregnancy. Although these findings may appear to be opposed, closer inspection of the data suggests that the lower values observed in the Micheal et al. study were comparatively high and in accordance with the higher values reported elsewhere. Future researchers must be mindful to state explicitly how high and low values are dichotomised, and efforts should be made to draw comparisons with other studies. Indeed, it is surprising, given its clinical significance, that studies failed to contrast the actual cortisol values, not just the pattern of the cortisol values and IVF association, with other published studies in this area. Indeed, drawing comparisons between studies may help better understand the point, or threshold, at which cortisol becomes potentially deleterious or conducive to IVF success.

Taken together, our review suggests that the quality of the available evidence for the role of cortisol in relation to IVF treatment is limited, with eleven studies considered to be low-quality publications. A number of factors which may account, at least in part, for the heterogeneity found within the literature have been discussed, and several methodological factors have been identified as potential sources of variance in this body of work. We encourage researchers to use our review to inform the design of future studies, taking particular attention to report essential extraneous factors associated with cortisol research in the context of IVF.

Future directions

It is apparent from this systematic review that several methodological limitations require further attention within the area of cortisol and IVF research. Indeed, future researchers should be mindful of how they conceptualise the stress process and the assumptions which are made when designing studies to best capture the stress response process. Study protocols that account for all stages of the stress process, so called 'high intensity' protocols, and optimally capture how cortisol may differ throughout different stages of an IVF cycle may help to better understand when during the course of an IVF treatment cycle chronic HPA axis activation may exert an effect. This would provide clinicians with a better understanding of when during an IVF cycle preventative stress interventions may be implemented with optimal effect.

Research which investigates the effects of chronic HPA axis activation over longer periods of time may also prove fruitful. Indeed, the evidence included in this review is based upon the assessment of cortisol within the time frame of the treatment process and, as such, offers a snap shot of short term activation albeit at different stages of a 6-week treatment process in some studies. To date, blood, saliva, follicular fluid and urine have been predominantly used, but these methods may not indicate the long-term effects of stress exposure very well [36–38]. Thus, we encourage future researchers to explore the potential advantages of other cortisol collection methods which are gaining popularity within stress research such as hair sampling. Hair sampling is a relatively new and unused sampling method within the IVF literature to date. Hair sampling methods may be used to obtain a measure of patient stress up to three months prior to the onset of a stressor. Whereas blood, saliva, follicular fluid, and urine capture real-time levels, hair cortisol analysis provides a complementary means of monitoring stress and capturing systemic cortisol exposure over longer periods of time. Indeed, this novel approach may prove a useful method capable of answering clinical questions relating to the cortisol and IVF relationship that could not previously be answered by other tests alone [39–41].

Strengths and limitations

This is the first systematic review to synthesise research on the role of cortisol in relation to IVF treatment outcomes; in doing so, our review complements two other related systematic reviews conducted on the role of negative effects/psychological stress and IVF treatment outcomes [2, 3]. Our review adds to the available evidence by examining the biological concomitant of stress rather than examining self-reported stress per se. A further advantage is that robust methods were used throughout the review process, and quality evaluations were made in accordance with standard protocols for all studies. However, studies included in this review were considerably heterogeneous, and thus it was not possible to use a meta analytic approach. Despite these challenges, our findings suggest that there is inconclusive evidence that cortisol plays a role in determining clinical pregnancy, oocyte number, oocyte fertilisation, oocyte cleavage, and miscarriage rates in patients undergoing IVF treatment, and our review

provides researchers with directions for future research and an overview of the methodological issues which require further attention in order to improve the quality of research in this area. Indeed, whilst the studies included in our review were considered low quality, we believe that the low quality and evident lack of scientific rigour aligns well with and emphasises why a robust systematic review is needed in this area. An important step for future research will be to address the methodological limitations discussed in our review and to consider how systemic, as well as short-term stress exposure, may exert an effect on IVF treatment outcomes.

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