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Comparative effectiveness of sildenafil citrate and estradiol valerate as adjuvants during clomiphene citrate-assisted ovarian stimulation cycles in patients with unexplained infertility: a double-blind randomized controlled trial

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Abstract

Objective This study aimed to assess the effect of sildenafil citrate and estradiol valerate as adjuvant therapy during ovarian stimulation cycles with clomiphene citrate in patients with unexplained infertility in Kisangani.

Method A double-blind, randomized controlled trial was conducted for two years at two specialized health facilities in Kisangani (University Clinics of Kisangani and "Clinique des Anges Kisangani"). The population included 148 patients, 74 of whom were on clomiphene citrate + sildenafil citrate (CCSC) regimens and 74 of whom were on clomiphene citrate + estradiol valerate (CCEV) regimens for three months. The primary indicator was the conception rate, with secondary outcomes encompassing endometrial thickness, appearance and vascularity, the number of mature follicles and ovulation rate.

Results The two groups were comparable in terms of sociodemographic and clinical characteristics. The mean duration of attempting to conceive was 4.39 years versus 4.36 years ($P=0.839$), while the mean AFC was 11.51 versus 11.46 ($P=0.831$), in the CCSC group versus CCEV group respectively. Secondary infertility was the most frequent diagnosis in each of the two groups. The biochemical pregnancy rate was comparable between the two groups ($P=0.385$), while the clinical pregnancy rate was significantly higher in the CCSC group versus CCEV group ($P=0.04$). Both perifollicular flow and the ovulation rate were significantly higher in the CCSC group versus the CCEV group ($P=0.006$ and $P=0.002$ respectively). However, endometrial vascularity/thickness, and the number of Graafian follicles were not significantly different between the two groups.

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Conclusion As an adjuvant, sildenafil increases the rate of clinical pregnancy more than does estradiol in patients with unexplained infertility undergoing ovarian stimulation with clomiphene citrate.

Study registration PACTR 202,310,849,449,401 (Pan African Clinical Trials Registry).

Keywords Unexplained infertility, Clomiphene ovulation, Sildenafil citrate, Estradiol valerate, Endometrial thickness

Introduction

The management of infertility is primarily aimed at correcting etiological factors. In the absence of a known correctable disorder, the treatment of unexplained infertility poses a difficult challenge. Infertility is considered unexplained when standard evaluation of the infertile couple has failed to identify an underlying etiological factor. In accordance with the World Health Organization (WHO) criteria [1], the standard assessment included documentation of regular ovulation, a normal uterine cavity, fallopian tube patency and a normal spermiogram.

The formulation of a treatment regimen is empirical [2] and must consider the efficacy, safety, risk, and costs of the various therapeutic options, as well as the age of the patient and her partner, the duration of their attempt to conceive, and cultural habits. The conventional approach to the treatment of unexplained infertility is to start with the least expensive and least invasive means before attempting advanced assisted reproductive technologies (ART) [3]. In accordance with the recommendations set forth by the European Society of Human Reproduction and Embryology (ESHRE), ovarian stimulation with intrauterine insemination is recommended as the first line of treatment for unexplained infertility [1].

Ovulation-inducing agents include clomiphene citrate (CC), aromatase inhibitors, and gonadotropins. Gonadotropin treatment is very expensive, less convenient (injectable), and associated with a high risk of ovarian hyperstimulation syndrome (OHSS) and multiple pregnancies. Oral ovulatory agents (CC and aromatase inhibitors) have relatively comparable efficacy [4]. However, widespread use of aromatase inhibitors for the management of unexplained infertility has not yet been approved by the Food and Drug Administration (FDA) [5].

CC is commonly used as a first-line treatment to induce ovulation due to its low cost, tolerability, and safety profile. Despite good ovarian stimulation and high ovulation rates, CC is associated with low pregnancy rates [6, 7] and high rates of early abortion [8–10]. The American Society of Reproductive Medicine (ASRM) Practice Committee has stated that CC does not increase fertility in patients with unexplained infertility [5]. This may be linked to the antiestrogenic effects of CC, notably the alteration of cervical mucus and endometrial receptivity [8–11].

Despite extensive research into the reliability of endometrial receptivity markers, endometrial thickness (ET) is still considered the most accurate surrogate measurement and a crucial component of implantation [12, 13]. Poor endometrial receptivity has been described as a major component of ART failure, and a direct link has been established between a thin endometrium (<7 mm) and low ART success rates [14]. To preserve or improve endometrial receptivity in patients undergoing ovarian stimulation with CC, several approaches have been proposed. These include the adjuvant use of estradiol [15, 16] and, more recently, sildenafil [17]. The effect of estrogen on ET during ovarian stimulation cycles with clomiphene citrate is the subject of much debate. Some studies have reported beneficial effects in the form of improved ET [18, 19], while others have shown that estrogen supplementation in clomiphene citrate-stimulated cycles has less of an effect on ET [20–22].

Endometrial growth depends on uterine blood flow. Steroid hormones, growth factors, integrins, and cytokines are involved in regulating endometrial development. Some of these factors are produced locally, while others must be transferred to the endometrium, which requires an adequate blood supply [23]. Sildenafil citrate (SC) is a selective inhibitor of phosphodiesterase type 5 (PDE5), the enzyme responsible for cGMP catabolism. This drug potentiates the relaxing effects of nitric oxide (NO) on smooth muscles by preventing the degradation of cGMP [24]. In this way, sildenafil promotes vasodilatation, a subsequent increase in uterine perfusion, and consequently an increase in ET. The effects of sildenafil on endometrial preparation for embryo transfer after in vitro fertilization (IVF) have been studied, and improvements in endometrial vascularization, thickness, and echogenicity have been noted [25, 26]. The literature shows that the addition of SC to CC improves the pregnancy success rate compared with the use of CC alone [17, 27].

The prevalence of unexplained infertility varies worldwide. Several studies have reported that in 10–40% of cases, the etiological factor of infertility remains unexplained [27, 28]. In the Democratic Republic of Congo (DRC), this prevalence is between 15% and 20% [29, 30]. However, little work has been done to compare the efficacy of sildenafil, a drug commonly prescribed to men with erectile dysfunction, with that of other adjuvants, such as estradiol, which is frequently used in Kisangani. Although IVF is widely recognized as an effective treatment for infertility [31], black patients are less responsive

to IVF treatment than Caucasians [32]. Moreover, in sub-Saharan Africa, particularly the DRC, IVF centers are rare and less accessible. In most cases, infertile couples are managed by more readily available treatment modalities, including CC [29]. A better understanding of how to prevent the adverse effects of CC on the endometrium and improve the success rates of CC will help to improve local clinical practices and update local guidelines for the management of couples' infertility. This study thus aimed to evaluate the efficacy of the oral administration of SC, compared with that of estradiol valerate (EV), as an adjuvant therapy to clomiphene citrate in improving ET and vascularization, ovulation rates, and pregnancy rates in patients with unexplained infertility. The province of Tshopo originates from the partition of the former Province Orientale, a region of the DRC that forms part of the infertility belt [33].

Methods

Study design

The study was a double-blinded, randomized, controlled trial that compared two ovulation induction regimens in patients with unexplained infertility. Coinvestigators and participants did not know which treatment the participants were receiving. Patients were randomly subjected to either a clomiphene citrate + sildenafil citrate (CCSC) or a clomiphene citrate + estradiol valerate (CCEV) regimen; they were recruited from two specialized health facilities in Kisangani, DRC: the University Clinics of Kisangani and ''Clinique des Anges Kisangani''. Patients with unexplained infertility were provided with explanations about the study (objectives, procedure, drugs used, their mechanisms of action, and adverse effects). Patients who consented to participate in the survey were then randomized into two groups using the permuted block randomization technique, with stratification according to duration of desire to conceive (≤2 years and >two years) and patient age (<30 years and ≥30 years). Randomization blocks of four were created using assignment sequences generated by an independent statistician, and the closed-envelope method was employed. Each envelope contained medication for three cycles of ovarian stimulation. Study participants were recruited and followed up from October 1, 2021, to October 31, 2023. The expected primary outcome in the present study was the conception rate, based on the occurrence of pregnancies (biochemical and clinical). Clinical pregnancy was defined by ultrasound visualization of one or more gestational sacs, whether normal or ectopic [34]. Secondary outcomes included ET, pattern, and vascularization; the number of mature follicles; PFBF; the stimulation cycle cancellation rate; the ovulation rate; and adverse events.

Patient recruitment criteria

Inclusion criteria

Duration of attempting to conceive for more than 1 year.

Age: 19–35 years.

BMI: 18.5–29.9 kg/m^2.

Regular menstrual cycles ranged from 21 to 35 days.

Evidence of good ovarian reserve, defined by the number of antral follicles per ovary, which ranged from 9 to 24 [35].

Evidence of bilateral tubal patency.

Normal husband's semen parameters (in accordance with WHO standards).

Informed consent.

Exclusion criteria

The presence of any etiological factor of infertility or abnormalities in the husband's semen analysis.

History of hormonal treatment within the past six months preceding the study.

History of allergy or presence of contraindications to the drugs utilized in this study.

Discontinuation of treatment or noncompliance with the total duration of the study (3 treatment cycles).

Histories of cardiac, hematological (hemoglobinopathy, etc.), renal, hepatic, metabolic (diabetes mellitus, hypothyroidism, etc.), or neurological disease.

Refusal to participate in the study.

Drug discontinuation criteria

Severe side effects.

Noncompliance with treatment regimen.

Withdrawal of informed consent.

Population sample

The sample size was calculated using a formula for estimating the difference in means between two independent samples [36]. For this purpose, we considered the effect of the study drugs on the endometrium, given that SC and EV are adjuvants used to minimize the negative effect of CC on ET.

$$n = \frac{\left(\delta_1^2 + \delta_2^2\right) \times \left(Z_{1-\alpha} + Z_{1-\beta}\right)^2}{\Delta^2}$$

where n = sample size, Z1-α = 1.96 for a two-tailed test for a first-species error α = 0.05 and a confidence level of 95%, and Z1-β = 0.84 for a power of 80% (β = 0.02). Δ (expected difference between the two groups, Δ = [μ1-μ2]). These studies included a randomized controlled trial previously published on the effects of SC and EV on endometrial receptivity [37, 38], Δ = 0.33 mm, σ1 (standard deviation of ET for exposed (treated with clomiphene citrate and sildenafil)) = 0.841 mm, and σ2 [standard deviation of ET for control group (treated with clomiphene and

estradiol)]=0.657 mm. Applying the above formula and estimating the proportion of patients lost to follow-up at 10%, the minimum sample size was deduced to be 144 patients, i.e., 72 patients in each arm.

Sample size

The procedure for enrolling participants is described in Fig. 1.

A total of 576 couples consulted the two institutions in which the present study was conducted in an attempt to conceive, and wives were assessed for eligibility. Of these, 428 were excluded (did not meet the inclusion criteria, refused to participate in the study), and 148 were included. The recruited patients were randomized into two groups: 74 participants were assigned to the CCSC regimen, while the remaining 74 were assigned to the CCEV regimen. During follow-up, no patient experienced severe side effects. However, 2 patients in the intervention group and 1 patient in the control group were removed from the analysis because they were lost to follow-up. In the CCSC group, 168 cycles were stimulated, while 171 cycles were stimulated in the CCEV group.

Intervention and follow-up

On admission, couples with a desire to conceive systematically underwent an etiological work-up for infertility, which included a clinical evaluation (medical history and physical examination) and a standard para-clinical work-up. This aetiological work-up was the same for both those who agreed to participate in the study and those who did not. The data collected in this study were prospective. In addition to sociodemographic data, the history of the male partner included pubertal development, history of

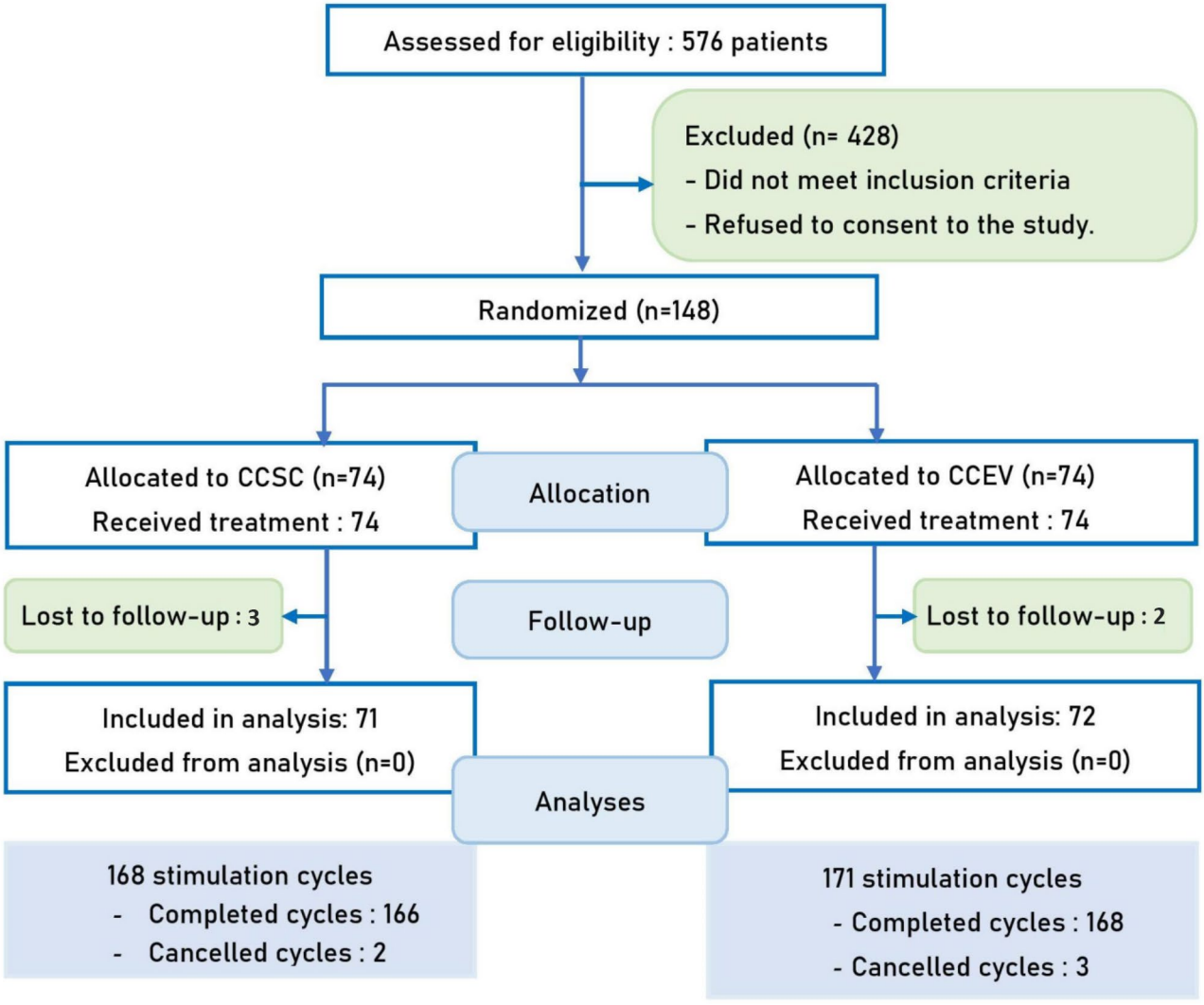

Fig. 1 Flow chart indicating the different steps of the study: enrollment of patients, allocation, follow-up, and analyses. *CC: clomiphene citrate, SC: sildenafil citrate, EV: estradiol valerate*

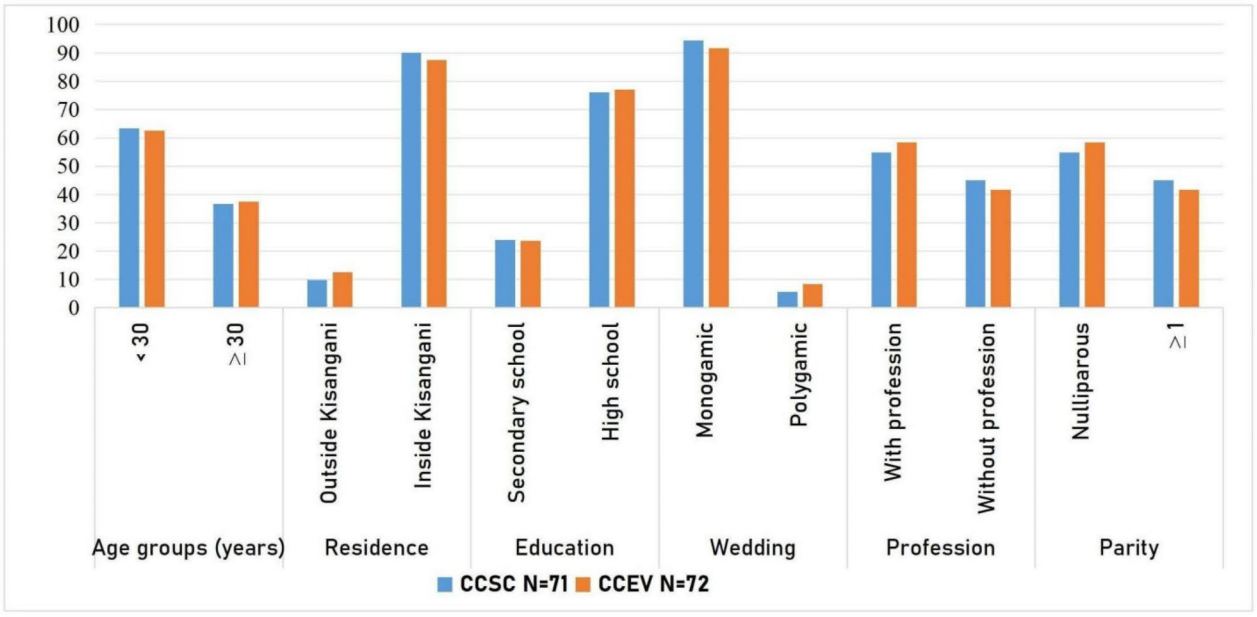

Fig. 2 Comparison of the demographic characteristics of women treated for unexplained infertilityy

cryptorchidism and testicular torsion, history of inguinal or testicular surgery, history of infection, medical history, previous paternity, previous treatment, etc. In women, their medical history focused on pubertal development, menstrual history, sexual history, gynecological and obstetrical history, medical history, and surgical history. The standard para-clinical work-up included ultrasound, hormone testing, tubal patency testing in the female partner, and a spermiogram in the male partner. Other para-clinical examinations were performed according to the clinical needs of each patient. When the couple's evaluation failed to identify an apparent aetiological factor for infertility, the couple was considered infertile due to unexplained causes.

The investigative team comprised 9 people, including a supervisor, four physicians, one medical biologist, one radiologist, and four nurses. Before the clinical trials, in addition to providing explanations of the data collection form, the investigators were trained in randomization and interview techniques, the pharmacology of the study drugs, participant follow-up, and the maintenance of the adverse event register. Physicians were also trained in the protocol for administering study drugs, monitoring ovarian stimulation, and investigating and managing adverse reactions to study drugs. Two nurses were trained to enrol patients in the study, while the other two were trained to assign patients to the different treatment groups. Medical biologists and supervisors were trained in the various biomedical procedures used in the trial.

Patients assigned to the CCSC intervention group received clomiphene citrate (50 mg tablet, 2x/day po, days 3 to 7 of the menstrual cycle) and sildenafil citrate (25 mg tablet, 2x/day po, days 8 to 12 of the same menstrual cycle). In comparison, patients in the CCEV control group received clomiphene citrate (50 mg tablet, 2x/day po, days 3 to 7 of the menstrual cycle) and estradiol valerate (2 mg tablet, 2x/day, days 8 to 12 of the menstrual cycle).

All treated patients underwent clinical and ultrasound follow-ups. Clinical follow-up was used to track the adverse effects of the drugs administered. Ultrasound follow-up consisted of follicular growth monitoring and endometrial assessment (thickness, echogenicity, and vascularization). All ultrasound scans were performed by a single operator, an obstetrician-gynecologist experienced in transvaginal sonography and trained in monitoring ovarian stimulation, who was not informed of the patient's randomization groups. The ultrasound machine used was an EDAN Acclarix AX8, version 1.2X, equipped with an 8 MHz transvaginal probe (Edan Instruments, Inc., 518122 Shenzhen, PR China).

Transvaginal ultrasound folliculography was performed every 24–48 h, starting on day 10 of the cycle. When at least one follicle had reached 18 mm in diameter, the ET and pattern were determined; the pulsatility index (PI) and resistance index (RI) of the uterine arteries, as well as perifollicular blood flow (PFBF), were measured using transvaginal Doppler ultrasound. Ovulation was then triggered with the intramuscular administration of 5000 IU chorionic gonatrophin, and the couple was advised to have scheduled intercourse.

The follicular diameter was defined as the mean of the two perpendicular diameters taken from the largest plane of the follicle [39]. When more than three mature follicles were detected, the patient was considered hyperstimulated. Under these conditions, the cycle was cancelled, and the couple was advised not to engage in unprotected intercourse during the ovulation window. During the next cycle, the hyperstimulated participants reduced the CC dose to 25 mg, 2x/day, and were monitored in the same way as the other participants. Endometrial thickness was measured on the longitudinal section of the uterus by the maximum distance between each myometrial and endometrial interface [40].

The endometrial pattern consisted of determining whether the endometrium was bi or trilamellar [39]. Uterine Doppler was performed on the ascending branch of the uterine arteries in segments located at the same level as the internal os of the cervix, and the indices (PI and RI) were automatically calculated. The PFBF was determined qualitatively using power Doppler imaging and classified into 4 grades based on the estimated percentage of perifollicular circumference in the perfusion map, representing vascularization and therefore blood flow. Grade 1 corresponded to blood flow ≤ 25% of the follicular circumference; Grade 2 corresponded to blood flow 26–50% of the circumference; Grade 3 corresponded to blood flow 51–75% of the follicular circumference; and Grade 4 corresponded to blood flow over 76–100% of the follicular circumference. Grades 1 and 2 were considered low-grade PFBF, while grades 3 and 4 were classified as high-grade perfusion [41–44]. To support the luteal phase, patients received dydrogesterone co 10 mg, 1 oral tablet per day for 14 days, starting on the day of ovulation induction. Subsequently, daily monitoring was performed until sonographic evidence of ovulation occurred. The criteria for ovulation were a sudden decrease in follicular diameter; the appearance of intrafollicular echoes; a scalloped follicular border; the replacement of the "triple line" appearance of the endometrium by a "homogeneous and hyperechoic" endometrium (luteinized endometrium); and the presence of a blade of free fluid in the cul-de-sac of Douglas [45].

Ovulation failure was diagnosed if no follicle had reached or exceeded 12 mm by cycle day 16, if the size of the follicle exhibiting dominance remained below 18 mm and did not progress for at least 72 h [40], or if the ultrasound criteria for ovulation remained absent even though the follicle had reached or exceeded 18 mm in diameter. Two weeks after ovulation induction, we performed a pregnancy test. When the pregnancy test was positive (biochemical pregnancy), the patient underwent ultrasound to confirm the pregnancy (clinical pregnancy). Successful treatment was defined by confirmation of clinical pregnancy. In the event of treatment failure, the patient resumed a new stimulation cycle on day 3 of the menstrual cycle with the same drug administration and monitoring protocol as the previous cycle, up to a maximum of three continuous cycles.

All drugs used in this study were subjected to strict quality control (Laboratoire d'Analyse et de Contrôle des Médicaments et des Denrées Alimentaires, "LACOMEDA" Kinshasa, DRC). The CC used in the study was branded Clomid® (Doppel Farmaceutici S.r.l., Italy) in a box of 10 tablets at a dosage of 50 mg each. Sildenafil citrate was purchased under the brand name Penegra® (Zydus Healthcare Ltd., India) in a box of 10 blister packs, each containing 4 tablets at 25 mg each. Estradiol valerate was purchased under the brand name Progynova® (Zydus Healthcare Ltd., India) in a box of 28 micronized tablets at 2 mg each. Dydrogesterone was purchased under the brand name Duphaston® (Abbot Biologicals BV, Netherlands) in a box of 10 film-coated tablets (2 mg each). Human chorionic gonadotropin was purchased under the brand name HUCOG®-5000HP (Bharat Serums and Vaccines Limited, India), and 5000 IU/1 ml vial was used.

Evaluation and management of side effects

During the study, participants were discouraged from self-medicating or using indigenous products, which are common practices in the DRC. Patients were provided with explanations of the adverse effects of the study drugs and were encouraged to report them. To enable standardized assessment by the investigators, an adverse event grid (appendix-1) and an objective assessment system were developed. In the objective evaluation system, the severity of adverse events was assessed as follows: 0 (symptoms not reported by the patient), 1 (mild symptoms not affecting lifestyle), 2 (moderate symptoms affecting lifestyle but controlled with simple means), and 3 (severe symptoms justifying immediate discontinuation of treatment and requiring hospital care for the patient).

Any other event reported by the participants but not included in the study drug prospectus was notified for analysis by the Congolese National Pharmacovigilance Center to determine whether the reported event was attributable to medication intake. Participants with mild and moderate side effects were treated as outpatients, while those with severe symptoms were advised to stop using the study medication and were hospitalized in one of the two health facilities where the study was conducted. Halfway through the trial, data related to the side effects were reported to the Ethics Committee of Kisangani University for approval to continue the trial.

Laboratory examination

Hormone assays were performed using Finecare™ (Guangzhou Wondfo Biotech Co., Ltd., PR China), a

lateral flow fluorescence immunoassay technology using the sandwich immunodetection method. HSG was performed between days 7 and 12 of the menstrual cycle using the usual metal cannula system [46]. The spermiogram was performed and interpreted according to WHO recommendations [47].

Statistical analysis

The study data were processed using Epi Info™ software version 7.2.2.6. Endometrial thickness was used to calculate sample size, as SC and EV are adjuvants used to minimize the negative effects of CC on ET.

The primary aim was to compare the conception rate (clinical pregnancies) between the two treatment regimens used in the study. The clinical pregnancy rate is defined as the number of clinical pregnancies per 100 cycles of stimulation [34]. For this purpose, rather than taking into account the number of patients included in the study, the results of the study were determined by the number of ovarian stimulation cycles. To determine the number of stimulation cycles required to achieve one more clinical pregnancy with one assignment group compared with the other, the number needed to treat for benefit (NNB) was calculated.

Secondary outcomes (ET, echogenicity, and vascularization; number of mature follicles; perifollicular flow; ovulation rate; adverse events) were also determined by the number of stimulation cycles. The frequency, proportion, mean, and standard deviation were calculated. To compare proportions, Pearson's $\chi 2$ test at a significance level of $P<0.05$ was used. When the conditions for applying Pearson's $\chi 2$ test were not met, Fisher's exact test at a significance level of $P<0.05$ was used.

To measure the strength of association between categorical variables, the risk ratio (RR) with its 95% confidence interval (CI) was determined. To compare means, Student's t test was performed using the pooled method or the Satterthwaite method, depending on whether the variances were homogeneous. To test the homogeneity of variances between the two assignment groups, the Bartlett test was used at a significance level of $P<0.05$.

To estimate the number of stimulation cycles required for an additional patient to experience an adverse event in one randomization group compared with the other, we calculated the number needed to treat for harm (NNH).

Ethical considerations

Study registration: PACTR 202,310,849,449,401.

The current study was conducted in accordance with the Declaration of Helsinki. The study protocol received approval from the ethics committee at the University of Kisangani (Ref. UNIKIS/CER/08/2021). Confidentiality safeguards were ensured through the implementation of anonymity throughout the various phases of data collection, processing, and analysis to uphold safety measures. The objectives and procedure of the study were explained to the patients beforehand. All participants signed an informed consent form before enrolling in the study.

Nomenclature statement of targets and ligands

The key targets and ligands mentioned in this article are permanently archived in «The Concise Guide to Pharmacology 2021/22» [48].

Results

Basic patient characteristics at randomization

Figure 2 describes the participants' sociodemographic characteristics. The two randomization groups (CCSC versus CCEV) were comparable in terms of participants' age (29 versus 28.8 years), level of education, type of marriage, occupation, and parity. The two randomized groups were also comparable in terms of their clinical characteristics (Fig. 3).

Table 1 describes the biological profile (basal serum hormone levels). No significant difference was observed between the two randomization groups.

Comparative treatment outcomes of women treated for unexplained infertility

Table 2 shows the study's treatment outcomes. The conception rate was determined based on the number of completed ovarian stimulation cycles (full cycles). A total of 339 cycles were stimulated in the 143 participants selected for analysis (71 assigned to CCSC and 72 assigned to CCEV). Of these 339 stimulation cycles, 334 (98.5%) were completed, and 5 cycles (1.5%) were cancelled due to an exaggerated response (more than 3 mature follicles).

The biochemical pregnancy rate was comparable between the two randomization groups (29.52% versus 27.38%, $P=0.333$), while the clinical pregnancy rate was significantly higher in the CCSC group versus the CCEV group (28.92% versus 20.83%, $P=0.04$). Indeed, the proportion of biochemical pregnancies that progressed to clinical pregnancies was significantly greater in patients assigned to the CCSC (48/49; 97.96%) than in those assigned to the CCEV (35/46, 76.09%) ($P=0.000$; RR=6.93, 95% CI=1.05–45.73). The conception rate for each stimulation cycle is illustrated in Table 3.

The secondary outcomes of the study showed that ET was comparable between CCSC (10.51 mm) and CCEV (10.39 mm), with no significant difference. Endometrial appearance and vascularity were also comparable between the two assignment groups, as was the number of maturing follicles (Table 2). However, in the CCSC group, perifollicular flow was predominantly high-grade ($P=0.002$), the ovulation rate was greater ($P=0.006$), and

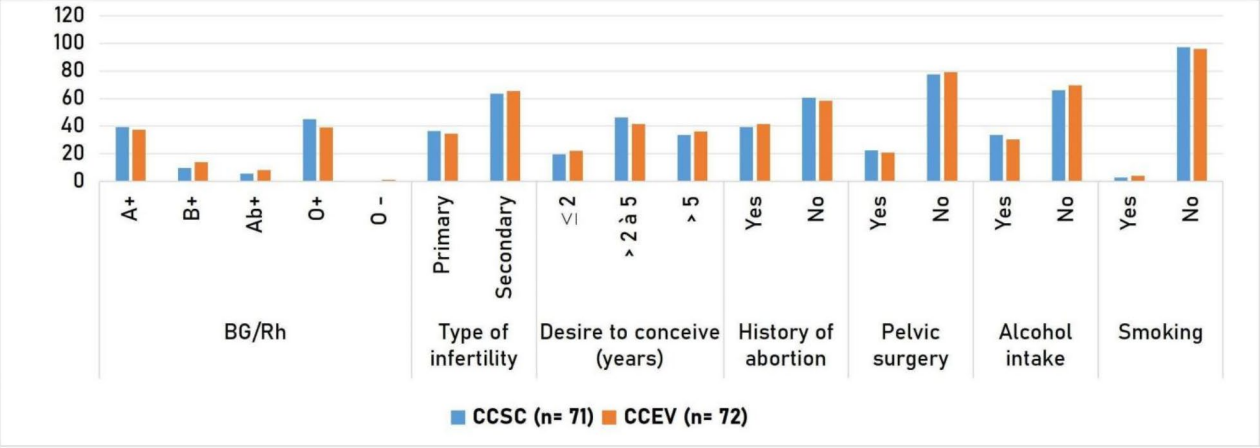

Fig. 3 Comparison of the clinical characteristics of women treated for unexplained infertility

Table 1 Comparative biological profile of women treated for unexplained infertility

Measurements	CCSC *n* = 71	CCEV *n* = 72	
	Mean ± SD	Mean ± SD	Student's t test
Menarche (years)	12.90 ± 1.22	12.89 ± 1.31	0.952
Menstrual cycle (days)	28.12 ± 1.49	28.45 ± 1.66	0.211
Coitarche (years)	18.09 ± 3.04	17.91 ± 2.70	0.706
BMI (kg/m2)	22.76 ± 1.13	22.46 ± 0.96	0.095
AFC	11.51 ± 1.49	11.46 ± 1.23	0.831
FSH (UI/L)	6.37 ± 0.75	6.45 ± 0.97	0.694
LH (mIU/L)	3.99 ± 0.77	4.06 ± 0.79	0.598
Estradiol (pg/ml)	57.55 ± 7.48	55.09 ± 8.37	0.066
AMH (ng/ml)	2.28 ± 0.47	2.31 ± 0.48	0.686

BMI: Body Mass Index, AFC: antral follicle count, FSH: follicle stimulating hormone, LH: Luteinizing hormone, AMH: anti-Müllerian hormone

ovulation was triggered slightly earlier (P=0.001) than in the CCEV group (Table 2).

Comparative side effects

As shown in Table 4, regarding the side effects of the study drugs, both treatment regimens were well tolerated, and no serious adverse events were reported by the participants. However, nausea was more common in the CCEV group (P=0.019), and headache was significantly more common in the CCSC group. The NNH for hot flushes with the CCEV protocol was 3. This means that with three cycles of stimulation with CCSC, one fewer patient experienced nausea than with stimulation with CCVE. For headache, the NNH was also 3 with CCSC, meaning that if three cycles were stimulated, one less patient would experience headache with CCEV than with CCSC. There was no difference in the incidence of ovarian hyperstimulation syndrome between the two randomization groups.

Discussion

The present study compared the effects of two adjuvant drugs (SC and VE) with CC on the endometrium, follicular development, ovulation rates, and pregnancy rates in patients with unexplained infertility. The two randomization groups had similar demographic and clinical characteristics. The results obtained provide evidence that ovulation induction with CCSC leads to biochemical pregnancy at rates comparable to those of CCEV in patients with unexplained infertility. However, the CCSC regimen favored the progression of biochemical pregnancies to clinical pregnancies in significantly greater proportions than did the CCVE regimen. The clinical pregnancy rate was 28.92% in the sildenafil group versus 20.83% in the VE group (P=0.04). This finding may suggest that the adjuvant use of SC significantly reduces the rate of very early miscarriage (subclinical abortions), which may be due to the effects of the drug on improving endometrial perfusion and receptivity.

Clomiphene citrate is a selective estrogen receptor modulator that, by inhibiting the negative feedback control of estradiol at the hypothalamic and pituitary levels, increases FSH secretion and consequently stimulates ovarian follicle development [5]. When used alone, CC leads to ovulation in 50–85% of cases, while the proportion of pregnancies achieved remains too low [6, 49]. This paradox can be explained in part by the antiestrogenic effect of CC, which reduces endometrial receptivity [49, 50] and generally results in implantation failure [51]. Certainly, the prolonged depletion of estrogen receptors caused by the antiestrogenic effect of CC has negative implications for both endometrial growth and development and the quality and quantity of cervical mucus.

Several adjuvant drugs have been used to prevent or minimize the antiestrogenic effects of CC, with varying results. These include estradiol [49], acetylcysteine [52,

Table 2 Comparative treatment outcomes within 3 months of ovarian stimulation cycles

Treatment outcome	Treatment regimens					
	CCSC *N* = 166		CC EV *N* = 168		*P* value	RR [95% CI]
Categorical variables	**n**	**%**	**n**	**%**		
Biochemical pregnancy						
Yes	49	29.52	46	27.38	0.333	1.05[0.83–1.33]
No	117	70.48	122	72.62		
Clinical pregnancy						
Yes	48	28.92	35	20.83	0.04	1.23[0.98–1.54]
No	118	71.08	133	79.17		
Ovulation occurrence						
Yes	163	98.19	155	92.26	0.005*	2.73 [0.98–7.62]
No	3	1.81	13	7.74		
Endometrial pattern						
No layering	2	1.21	3	1.79	0.342*	0.79[0.27–2.34]
Distinct 5-line appearance	142	85.54	141	83.93	réf.	1
Hazy 5-line appearance	22	13.25	24	14.28	0.385	0.95[0.68–1.31]
Endometrial vascularization						
Zone 1–2	6	3.61	10	5.95	0.166	0.74[0.39–1.41]
Zone 3–4	160	96.39	158	94.05		
Perifollicular blood flow						
Low-grade	36	21.69	60	35.71	0.002	0.68[0,51-0.91]
High-grade	130	78.31	108	64.29		
Gradual variables	**Mean ± SD**		**Mean ± SD**		**Student's t test**	
Endometrial thickness (mm)	10.39 ± 1.29		10.51 ± 1.61		0.481	
Day of ovulation triggering (n)	14.72 ± 1.04		15.10 ± 1.22		0.001	
Number of mature follicles (n)	1.53 ± 0.65		1.52 ± 0.69		0.897	

*CCSC = clomiphene citrate + sildenafil citrate, CCEV = clomiphene citrate + estradiol valerate, SD = standard deviation, RR = relative risk, CI = confidence interval, *Fisher's exact test*

Table 3 Conception rate per stimulation cycle

Treatment outcomes	CCSC				CCEV			
	1rst cycle (*N* = 70)	2nd cycle (*N* = 55)	3rd cycle (*N* = 41)	Total (*N* = 166)	1rst cycle (*N* = 70)	2nd cycle (*N* = 54)	3rd cycle (*N* = 44)	Total (*N* = 168)
	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)
Biochemical pregnancy	19(27.14)	17(30.9)	13(31.7)	49 (29.52)	19(27.1)	13(24)	14(31.8)	46(27.38)
Clinical pregnancy	19(27.14)	16(29.1)	13(31.7)	48(28.92)	15(21.4)	10(18.5)	10(22.7)	35(20.8)

N = number of stimulation cycles, n = number of patients who conceived, % = proportion of patients who conceived

Table 4 Side effects reported by randomization group

Side effects		Treatment regimens						
		CCSC *N* = 168		CCEV *N* = 171		FE	RR [95% CI]	NNH
		n	**%**	**n**	**%**			
Hot flushes								
	Yes	2	1.19	10	5.85	0.019	0.32[0.09–1.68]	3
	No	166	98.81	161	94.15			
Headache								
	Yes	18	10.71	3	1.75	0.000	1.82[1.47–2.24]	3
	No	150	89.29	168	98.25			
Mild hyperstimulation								
	Yes	2	1.19	3	1.75	0.508	0.80[0.27–2.36]	
	No	166	98.81	168	98.25			

CCSC = clomiphene citrate + sildenafil citrate, CCEV = clomiphene citrate + estradiol valerate, FE = Fisher's exact test, RR = relative risk, CI = confidence interval, NH = number needed to treat for harm.

53], nitroglycerin [54], vitamin E [55] and, more recently, sildenafil. Studies investigating the effects of SC on endometrial receptivity in patients with unexplained infertility have shown a significant increase in ET and pregnancy rate compared with placebo, regardless of the route of administration [6, 17, 56, 57]. Therefore, SC has been successfully used to achieve the same effects in patients with thin endometrium and to prepare the endometrium for embryo transfer in IVF cycles. Similar results have been reported in studies comparing the adjuvant use of EV with placebo in ovarian stimulation cycles with CC [15].

However, our study revealed no significant differences between the two treatment regimens (CCSC versus CCEV) with respect to ET, pattern, or vascularization. Mangal et *al.* [58] and Ali Dawood et *al.* [37] compared the effects of vaginal sildenafil and estradiol valerate on ET and the conception rate in infertile patients with thin endometrium. These studies revealed no significant difference in ET between the two randomized groups. However, the pregnancy rate was significantly greater in the CS group than in the EV group. These results thus suggest that in addition to ET, SC may have other mechanisms to promote egg implantation and pregnancy progression. SC improves PFBF [44] and reduces natural killer (NK) cell activity in the peripheral blood [59, 60]. There is evidence of an association between PFBF and oocyte quality [43, 44, 61]. In the present study, PFBF was predominantly high-grade (75–100%) in the CCSC group than in the CCEV group. NK cell activity was not assessed in this study. Another way in which sildenafil may improve pregnancy rates is by relaxing the myometrium. Uterine contractions affect implantation, probably by mechanically displacing the embryo. Decreases in pregnancy and implantation rates have been observed as the frequency of uterine contractions increases [62, 63].

Another important piece of data to emerge from this study relates to ovulation. The number of mature follicles was comparable between patients receiving CCSC and those in the control group. However, the ovulation rate was significantly greater, and ovulation was triggered earlier in the CCSC group than in the control group. Headache was significantly more common in the CCSC group than in the CCEV group. Headache has been reported by others [17, 64] and is thought to be related to the vasodilatory effect of sildenafil. Other adverse effects frequently reported in the literature in connection with the CCSC combination, such as hypotension and tachycardia, were not observed in the present study.

Study limitations

One of the strengths of the present study is that it compared the effects of the adjuvant use of CS with those of another adjuvant (EV). There is extensive scientific literature on the efficacy of SC as an adjuvant in ovarian stimulation cycles with CC. However, most comparisons have generally been with placebos [17, 56, 57]. Compared with VE, another adjuvant also recognized as effective in improving endometrial receptivity, SC may be a good alternative, especially because CS is widely available and less expensive in our circulation. The other strength relates to the methodology used.

Double-blind randomization limits the influence of investigators on the study results. In addition, the fact that we compared two homogeneous groups of patients with similar sociodemographic and clinical characteristics and for whom unexplained infertility was the only indication for treatment meant that the results obtained can only be attributed to the effects of the drugs alone. Weaknesses include the lack of long-term follow-up of patients who became pregnant. This makes it impossible to determine the long-term effects of sildenafil citrate on pregnancy and fetal outcomes.

Conclusion

Sildenafil, like estradiol, can be used to protect the endometrium from the adverse antiestrogenic effects of CC. However, compared with the use of EV, the adjuvant use of SC increases the clinical pregnancy rate in infertile patients with unexplained infertility undergoing ovarian stimulation with CC. In addition to improving endometrial receptivity, improving follicular perfusion contributes to the production of high-quality oocytes, providing another pathway by which sildenafil can support the progression to clinical pregnancy. We thus recommend the routine use of SC in patients with CC. However, multicenter studies with larger numbers of patients are needed to prove the value of this method in improving the results of ovarian stimulation in patients with CC.

Abbreviations

AFC	Antral follicle count
AMH	Antimüllerian hormone
BMI	Body mass index
CC	Clomiphene citrate
CCSC	Clomiphene citrate + Sildenafil citrate
CCEV	Clomiphene citrate + estradiol valerate
CI	Confidence interval
DRC	Democratic Republic of the Congo
ERA	Endometrial Receptivity Array
EV	Estradiol valerate
FSH	Follicle-stimulating hormone
IVF	In Vitro Fertilization
LACOMEDA	Laboratory for Analysis and Control of Medicines and Foodstuffs
LH	Luteinizing hormone
PI	Pulsatility index
PRL	Prolactin
RR	Relative risk
SC	Sildenafil citrate
SD	Standard deviation
WHO	World Health Organization

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Author contributions
J.D.B.N. conceived the protocol; R.M.D. and J.J.SKV. revised it; A.M.O and G.K.B. validated it; J.D.B.N., J.J.SKV and A.M.O. supervised the entire study from realization to data collection and data treatment; J.D.B.N, N.L.O., K.J.T and A.H.B.C. wrote the manuscript; A.M.O., R.M.D and G.K.B. checked the final version of the manuscript. All the authors approved its final version.

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Data availability
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
The study received ethical clearance from the research ethics committee of the University of Kisangani (Ref. UNIKIS/CER/08/2021). Participation was free and anonymous, based on written consent. Refusal to participate in the study did not impact the infertile couple's access to appropriate care.

Consent for publication
Consent signed by participating patients allowed us to disseminate anonymised study findings through publications.

Competing interests
The authors declare no competing interests.

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