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Original Article

Obstetrics and Gynaecology Section

Comparison of the Pharmacokinetics, Bioequivalence and Safety of Aqueous Progesterone Formulation Administered as either Intramuscular or Subcutaneous Injection versus Oil-based Progesterone Formulation Administered as Intramuscular Injection: A Randomised Study

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ABSTRACT

Introduction: Progesterone is the treatment of choice for support of the luteal phase of controlled ovarian stimulation cycles in women undergoing an Assisted Reproductive Technology (ART) treatment. Available progesterone preparations include oral, vaginal and oil-based Intramuscular (i.m.) formulations. Oral formulation have poor bioavailability whereas vaginal formulations cause side-effects such as vaginal discharge and/or local irritation. Oil-based progesterone formulations for i.m. use are associated with discomfort and pain at the injection site. Hence, a novel aqueous-based progesterone formulation for i.m./Subcutaneous (s.c.) was developed to avoid the local tolerability issues of the existing parenteral formulations.

Aim: To assess single-dose Pharmacokinetics (PK) and relative bioavailability of i.m. (test-1; T1) or s.c. (test-2; T2) administration of novel aqueous progesterone formulation with i.m. (reference; R) administration of oil-based progesterone formulation.

Materials and Methods: In this open-label, three-sequence, three-period, single-dose, cross-over study, 51 healthy human postmenopausal female subjects between 45 to 65 years of age were included. The study was conducted at Lambda Therapeutic Research Limited, Ahmedabad, Gujarat, India, between 21 May 2018 to 06 July 2018. Subjects were randomised to a single 25 mg dose of T1, T2 or R in three-periods (Period-I: T1, R, T2; Period-II: T2, T1, R; Period-III: R, T2, T1) with ≥18 days washout period. Blood samples were collected at prespecified time points in each period and analysed using validated liquid chromatography with tandem mass spectrometry. PK parameters {maximum plasma concentration (C_{max}), time to reach C_{max} (T_{max}), area under the plasma concentration vs. time curve (AUC_{0-t}), AUC from time 0 to ∞ ($AUC_{0-\infty}$), plasma half-life ($t_{1/2}$)} were calculated from the plasma concentration vs. time profile by non compartmental model. The total study duration was about 47 days (11 hours prior to the drug administration in Period-I until the last ambulatory sample in Period-III). All patients provided written informed consent form and an approval from the Conscience-Independent Ethics Committee (CIEC) was taken. Descriptive statistics were calculated and reported for PK parameters for baseline corrected and uncorrected data.

Results: Of 72 screened patients, 51 patients were included for the PK and statistical analysis. The mean±SD age of the patients was 55.1±4.67 years. The baseline corrected PK data shows that in T1, T2 and R arms, mean (range) T_{max} were 1.00 (0.50–1.75), 1.00 (0.75–1.75) and 8.00 hours (1.00–12.00), mean±SD $t_{½}$ (h) were 15.43±5.81, 15.27±6.68 and 19.80±6.35; mean±SD C_{max} (ng/mL) were 101.91±73.07, 51.67±14.81 and 18.89±7.89, and mean±SD AUC_{0-t} (ng/mL) were 385.10±89.29, 349.63±64.41 and 371.50±56.25, respectively. Similarly, the $AUC_{0-\infty}$ was also comparable in all three arms. The baseline uncorrected data were also in line with baseline corrected data. For AUC_{0-t} and $AUC_{0-\infty}$, 90% CIs were 98.44-107.06% and 97.96-106.15%, respectively, for T1/R ratio, and 90.01-97.90 and 89.90-97.42, respectively, for T2/R ratio. Six Adverse Events (AEs) in four subjects were reported. All AEs were mild in nature and there were no deaths, significant or serious AEs reported. Overall, all the treatments were well-tolerated without any new safety concerns.

Conclusion: Novel aqueous progesterone formulation i.m./s.c was bioequivalent with oil-based progesterone formulation i.m. with respect to AUC. The s.c. administration of aqueous progesterone formulation could offer a convenient alternative to the i.m. oil-based progesterone formulation for luteal phase support to patients undergoing ART treatments.

Keywords: Aqueous formulations, Bioavailability, Oil-based formulations, Progesterone

INTRODUCTION

Progesterone is an important endogenous steroid hormone that regulates the female reproductive function, ovulation and menstruation, required for implantation and maintenance of early pregnancy [1,2]. It is the treatment of choice for luteal phase support in women undergoing ovarian stimulation in In Vitro Fertilisation (IVF)/Intracytoplasmic Sperm Injection (ICSI) cycle as part of an ART treatment, and is associated with high rates of live birth or ongoing pregnancy [3,4]. Available progesterone preparations are oral, vaginal and injectable formulations [5].

The oral formulations have the advantage of ease of administration but has the limited use in infertility considering their poor bioavailability

due to its rapid clearance by first-pass hepatic metabolism [6,7]. Also, studies have reported a lower efficacy of orally administered progesterone versus intramuscular (i.m.) or vaginal formulations in terms of pregnancy rate when used for luteal phase support in ART [8]. For achieving high pregnancy rates, oral progesterone formulations require high doses, which are associated with systemic side-effects [9].

Vaginal formulations provide adequate luteal phase support however they are associated with vaginal discharge and local irritation [10-12]. Progesterone administered as i.m. injection reliably achieves serum levels of progesterone encountered in the menstrual cycle luteal phase but can cause patient discomfort, pain, inflammatory reaction at the injection site, sterile abscesses, and possible infection. The oil-based progesterone formulations are administered as intramuscular (i.m.) injection and causes pain and discomfort at the injection site [9,13].

Hence, a novel aqueous based progesterone formulation 'Lubion' was developed to avoid the local tolerability issues of the existing parenteral formulations. This aqueous progesterone formulation can be administered via s.c. route, and provides the benefits of precise dosing of the injectable formulation and avoids the pain associated with oil-based progesterone formulations. The PKs, efficacy and safety of this novel aqueous based progesterone formulation have been well established, and Lubion is approved and available in the United Kingdom [14,15].

Intas pharmaceuticals limited has developed a similar aqueous-based progesterone formulation in India, which could be administered via both i.m. and s.c. routes. The data on PKs of aqueous progesterone formulation administered via s.c. or i.m. routes in comparison with oil-based progesterone formulation administered via i.m. route is limited. Hence, this study was conducted with an aim to evaluate the PKs and relative bioavailability of Intas' aqueous-based progesterone formulation (Progesterone solution for injection 25 mg/1.119 mL Vial) administered via s.c. or i.m. routes in comparison with the oil-based progesterone formulation (Gestone, Progesterone injection IP 50 mg/mL) administered via i.m. route. Thus, the aim of the study was to assess single-dose PKs and relative bioavailability of i.m. (test-1; T1) or s.c. (test-2; T2) administration of novel aqueous progesterone formulation with i.m. (reference; R) administration of oil-based progesterone formulation.

MATERIALS AND METHODS

The open-label, three-sequence, three-period, single-dose, cross-over study was conducted between 21 May 2018 and 06 July 2018 at Lambda Therapeutic Research Ltd., (Ahmedabad, Gujarat, India). The study was conducted in accordance with the pertinent requirements of the Schedule Y (with subsequent amendments) of Central Drugs Standard Control Organisation (CDSCO), Ministry of health and family welfare, Government of India, 'National Ethical Guidelines for Biomedical and Health Research Involving Human Participants', Indian Council of Medical Research (ICMR 2017), ICH (The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) E6 (R2) 'Guideline for Good Clinical Practice' 2016 and Declaration of Helsinki (Brazil, October 2013) [16]. The study protocol and the informed consent form were approved by the Conscience-Independent Ethics Committee (CIEC; ECR/233/Indt/GJ/2015).

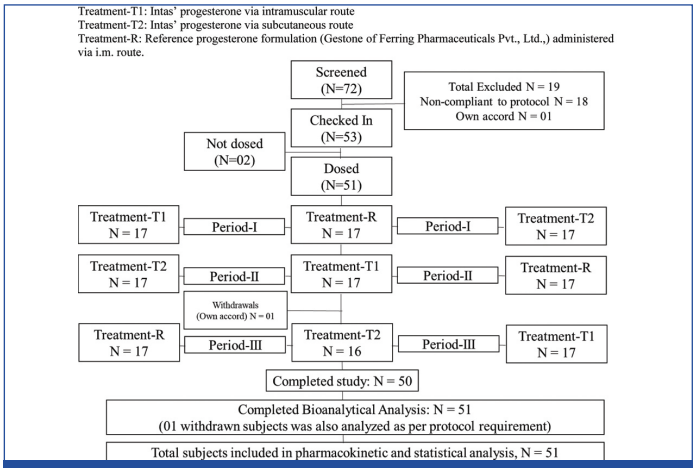
Sample size determination: A sample size of 51 subjects was determined using SAS® by considering the assumptions of T/R ratio (85.0-117.6%) and intrasubject variability (~11%, based on literature), significance level (5%), power (≥80%) and bioequivalence limits (80.00-125.00%) for 90% CI, dropouts and/or withdrawals [17].

Subjects: The study subjects were healthy postmenopausal female volunteers aged between 45 and 65 years (both inclusive) with body mass index of 18.5-30.0 kg/m^2 [18]. Eligibility of subjects was determined by clinical examination, vital signs {sitting blood pressure (≥110/70 mmHg and ≤140/90 mmHg), radial pulse rate (>60 or <100 beats per minute), oral body temperature (Fahrenheit) and respiratory rate (per minute)}, clinical laboratory evaluations (haematology, biochemistry and urine analysis), 12-lead electrocardiography, chest X-ray (posterior-anterior view), immunological tests, pap smear, mammography (within last one year), estimation of serum Follicle Stimulating Hormone (FSH) and estradiol level, gynaecological examination and medical history.

Inclusion criteria: Postmenopausal female volunteers who had a negative serum pregnancy test, 12 months of spontaneous amenorrhoea or six months of spontaneous amenorrhoea with serum FSH levels >21 mIU/mL or six weeks of postsurgical bilateral oophorectomy with or without hysterectomy, having a clinically acceptable serum FSH and estradiol levels, and Pap smear and mammography report were included in the study.

Exclusion criteria: Patients with known hypersensitivity to progesterone or any excipients or any related drug or any substance, receiving hormone replacement therapy or depot injection or implant of any drug within three months and significant illness that preclude their participation were excluded from the study.

Study design and blood sampling: The subjects were assigned to one of three sequence groups, using a randomisation schedule. This was a single dose bioequivalence study. Subjects were administered a single dose of either i.m. (test product 1-T1) or s.c. (test product 2-T2) injection of test formulation or i.m. injection of reference (R) formulation in each period [Table/Fig-1]. The study was divided into Period-I; 21 May 2018 to 31 May 2018, Period-II; 08 June 2018 to 18 June 2018 and Period-III; 26 June 2018 to 06 July 2018.



[Table/Fig-1]: CONSORT diagram for patient disposition.

A washout period of atleast 18 days was maintained between two consecutive dose periods based on t1/2 values of progesterone. The maximum t1/2 reported after s.c. administration of aqueous progesterone formulation is 43.4 hours, hence, a washout period of atleast 18 days was considered to avoid any carryover effects. The Total study duration was about 47 days (11 hours prior to the drug administration in Period-I until the last ambulatory sample in Period-III).

The study participants were housed in Lambda's clinical facility atleast 11 hours before administration of the Investigational Medicinal Product (IMP) and continued to remain in the clinical facility for atleast 48 hours after administration of the IMP in each period. During each period, after an overnight fast of ≥10 hours, a single 25 mg (1.119 mL) intramuscular (test product 1-T1) or subcutaneous (test product 2-T2) injection of test formulation (progesterone solution for injection 25 mg/1.119 mL vial; manufactured by Intas Pharmaceuticals Ltd., India; batch# X48006; expiry date 01/2020), or single 25 mg (0.5 mL) intramuscular injection of reference formulation {R; Gestone (progesterone injection IP 50 mg/mL); marketed by Ferring Pharmaceuticals Pvt., Ltd., Mumbai, India;

batch# F63703H; expiry date 02/2020} was administered to the subjects in supine posture and subjects remained in the supine position for ≥15 minutes following drug administration. Subjects were instructed to abstain from any xanthine containing foods or beverages (like tea, coffee, chocolates or cola drinks), tobacco or tobacco containing products (like pan, pan masala, gutkha), beedi and cigarette for 48 hours prior to drug administration in each period and throughout their stay in the clinical facility. Further, they were instructed to abstain from grapefruit or grapefruit products (within 72 hours prior to drug administration in Period-I till last PK sample of Period-III), alcohol or alcoholic products and recreational drugs (within 48 hours prior to drug administration in Period-I till last PK sample of Period-III) and unusual diet (fasting, high potassium or low-sodium) (within four weeks prior to drug administration in Period-I till last PK sample of Period-III) as these may alter the PKs of the drugs.

Blood samples of 4.5 mL were drawn by the trained study personals through an indwelling intravenous cannula (Venflon) placed in the forearm vein. Cannula was removed after 24.00 hour postdose sample and the subsequent samples were collected through fresh vein puncture. In the T1 and T2 arms, the blood samples were withdrawn at predose {-1.00, -0.50, 0.00 hour (within 5 minutes before dosing)}, 0.167, 0.333, 0.500, 0.750, 1.000, 1.250, 1.500, 1.750, 2.000, 2.333, 2.667, 3.000, 3.500, 4.000, 5.000, 6.000, 8.000, 12.000, 18.000, 24.000, 48.000, 72.000, 120.000, 168.000 and 216.000 hours following drug administration in each period. In reference formulation arm, blood samples were withdrawn at predose {-1.000, -0.500, 0.000 hours (within 5 minutes before dosing)} and at 1.000, 2.000, 3.000, 4.000, 5.000, 6.000, 6.500, 7.000, 7.500, 8.000, 8.500, 9.000, 10.000, 11.000, 12.000, 14.000, 16.000, 20.000, 24.000, 36.000, 48.000, 72.000, 120.000, 168.000 and 216.000 hours following drug administration in each period. Blood samples at and after 72.00 hours postdose were collected on ambulatory basis in each period. The blood samples were centrifuged at 3000±100 rcf for five minutes at 2°C to separate plasma, which were transferred to prelabeled polypropylene tubes in two aliquots and stored at a temperature -65±10°C until completion of analysis by liquid chromatography with tandem mass spectrometry (LC-MS/MS).

Safety assessments: Safety assessments consisted of monitoring and recording of AEs such as diarrhoea, vomiting, eosinophilia, dizziness, pyrexia etc., monitoring of clinical laboratory parameters (haematology, biochemistry and urine analysis), clinical examinations and monitoring of vital signs throughout out the study period. Clinical examinations were performed at screening, after check-in and before check-out of each period and at the end of study (after last ambulatory sample of Period-III). The vital signs (sitting blood pressure and pulse rate) were measured at screening, before dosing and at 1, 3, 6, 24 and 36 hours after dosing. Subjects were questioned for well-being along with clinical examination, vital signs assessment and ambulatory visit compliance assessment activity. During vital and clinical examination, subject's medical status was assessed based on clinical examination or vital parameter and subjects were asked about their health status. Further, ambulatory visits, compliance assessment such as restriction related to food, prohibitory item, medicines etc., were confirmed with subjects. Also, subjects were asked about their health status and their responses were documented as part of well-being assessment. Laboratory tests were performed at screening, and at the end of study. Serum pregnancy tests were performed on screening, prior check-in of each period and at the end of study. All laboratory tests including serum pregnancy test) were performed at Lambda Therapeutic Research Ltd., Ahmedabad, Gujarat, India. All AEs, including both observed and reported problems/complaints, signs or symptoms occurring after the dose administration were recorded regardless of any suspected relationship to study drug. Safety assessments were performed till end of the study, i.e., the last ambulatory sample of Period-III.

Determination of plasma concentrations of progesterone: A team of study personnel (analysts) were involved in the sample analysis were kept blinded from the randomisation code during the entire study/analysts were blinded to the randomisation scheme. An appropriate LC-MS/MS method was developed and validated at the Bioanalytical facility of Lambda Therapeutic Research Ltd., Ahmedabad, India. The plasma samples were analysed using this validated LC-MS/MS method for progesterone at Lambda Therapeutic Research Ltd., Ahmedabad, India. Calibration curve using an 8-point calibration curve standard, with concentrations ranging from 0.151 ng/mL to 150.444 ng/mL [18] were used to determine the concentrations of progesterone.

Pharmacokinetic (PK) and Statistical Analysis: The PK parameters were calculated from the plasma concentration vs. time profile by non compartmental model using Phoenix® WinNonlin® Version 6.4 (Certara L.P.) for baseline corrected and baseline uncorrected data of progesterone [18]. Baseline corrections were determined for each dosing period. The adjustments were performed by subtracting the mean baseline values from plasma concentrations for postdose samples (including predose at 0.000 hour) prior to the calculation of the PK parameters for progesterone. If a negative plasma concentration value resulted after baseline correction, it was set to zero (0) prior to the calculation of baseline corrected PK parameters. Baseline uncorrected PK parameters were estimated based on concentration data without any adjustment of the endogenous levels.

The maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were calculated from the plasma concentration vs. time profile of individual subjects. Area under the plasma concentration vs. time curve (AUC_{0-t}) was calculated by linear trapezoidal rule from measured data points from the time zero to the time of last quantified concentration [17]. AUC0-∞ was calculated as AUC0-t+$C_t/\lambda z$, where C_t is the last measurable concentration and λ_z is the terminal rate constant estimated via linear regression of time vs. log transformed concentration. The t½ was calculated as $0.693/\lambda_z$ [17].

STATISTICAL ANALYSIS

Descriptive statistics were calculated and reported for all the PK parameters for baseline corrected and baseline uncorrected data. The mean of -1.000, -0.500 and 0.000 hours predose levels were used for the period specific baseline adjustment of the postdose levels for progesterone, which was performed by subtracting this mean baseline from every plasma concentration for post dose samples. The natural logarithm (ln)-transformed PK parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were subjected to Analysis of Variance (ANOVA) for baseline corrected and uncorrected data of progesterone. ANOVA model included sequence, formulation and period as fixed effects and subject (sequence) as a random effect.

Statistical comparison of the PK parameters of the three arms were carried out using PROC MIXED of SAS® Version 9.4 (SAS Institute Inc., USA) to assess the comparison between two test arms and one reference arm. ANOVA, 90% Confidence Interval (CI) using two one-sided tests, power and ratio analysis were performed on ln-transformed PK parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ for both baseline corrected and baseline uncorrected data.

RESULTS

Demographic data: A total of 72 subjects were evaluated for enrolment, of which 53 subjects checked-in for the study but two of these patients were not dosed. Hence, a total of 51 healthy postmenopausal female subjects were dosed and considered for the PK and statistical analyses. Of these, 50 subjects completed the study, and one subject discontinued from Period-III in test arm (subcutaneous) of the study on her own accord [Table/Fig-1].

The demographic characteristics of the study population are summarised in [Table/Fig-2].

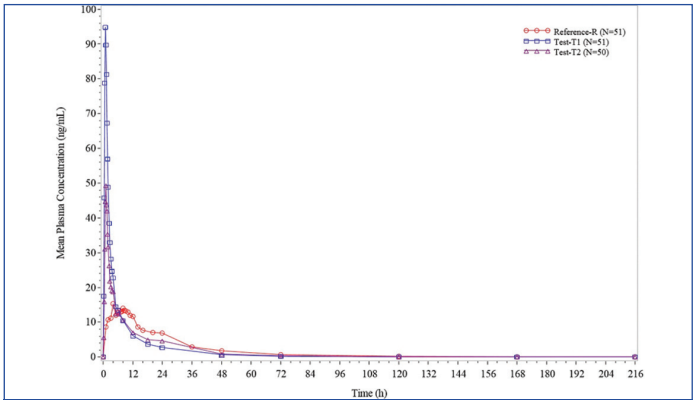
Characteristics	Mean±SD
Age (years)	55.1±4.67
Weight (kg)	59.42±6.95
Height (cm)	151.12±5.16
BMI (kg/m^2)	26.01±2.71

[Table/Fig-2]: Baseline demographic and clinical characteristics (n=51).
BMI: Body mass index; SD: Standard deviation

Safety: The tolerability of both formulations, administered as single dose, was acceptable. No serious AEs occurred in any of the three arms. A total of six AEs were observed in four subjects, with two AEs reported in each of the three arms (T1: diarrhoea and eosinophilia; T2: vomiting and dizziness; R: increased white blood cell count and pyrexia). All AEs were mild in nature. Of these six AEs reported, the causality assessment was judged as possibly related for two AEs and as unlikely related for four AEs. No clinically significant abnormalities in physical examination and vital sign measurements were reported.

Pharmacokinetic (PK) analysis: The mean plasma concentration-time curves of progesterone in all three arms are shown in [Table/Fig-3].

The descriptive statistics of PK parameters for baseline corrected and baseline uncorrected data are listed in [Table/Fig-4,5], respectively.



[Table/Fig-3]: Mean plasma concentration-time profiles of test 1, test 2 and reference arms.

Parameter (unit)	Test product-T1 (n=51), mean±SD; p-value	Test product-T2 (n=50), mean±SD; p-value	Reference product-R (n=51), mean±SD
C_{max} (ng/mL)	101.91±73.07 p<0.0001#	51.67±14.81 p<0.0001#	18.89±7.89
T_{max} (h)*	1.00 (0.50-1.75) p<0.0001^	1.00 (0.75-1.75) p<0.0001^	8.00 (1.00-12.00)
AUC_{0-t} (ng.h/mL)	385.10±89.29† p=0.1869#	349.63±64.41 p=0.0085#	371.50±56.25†
$AUC_{0-\infty}$ (ng.h/mL)	392.72±90.55† p=0.2379#	357.42±63.81 p=0.0033#	381.32±55.73†
t½ (h)	15.43±5.81† p=0.0002#	15.27±6.68 p=0.0003#	19.80±6.35

[Table/Fig-4]: Pharmacokinetic (PK) parameters of progesterone (baseline corrected data) in three arms.
Data presented as Mean±SD; PK parameters are computed from baseline corrected plasma drug concentration data.
*T_{max} is represented as median (min-max) value; †N=50.
Test 1: single 25 mg (1.119 mL) intramuscular injection of aqueous progesterone; Test 2: single 25 mg (1.119 mL) subcutaneous injection of aqueous progesterone; and Reference: single 25 mg (0.5 mL) intramuscular injection of oil-based progesterone formulation.
AUC_{0-t}, area under the curve from time 0 to t; $AUC_{0-\infty}$; area under the curve from time 0 extrapolated to infinite time; C_{max}, maximum plasma drug concentration; t½, plasma half-life; T_{max}, time to reach C_{max}.
#p-value is calculated using paired t-test for comparison for T1 vs R and T2 vs R.
^p-value is calculated using Wilcoxon signed rank test for comparison for T1 vs R and T2 vs R.
p-value <0.05 is considered statistically significant

Baseline corrected data: The mean (range) T_{max} (hr) in T1, T2 and R groups were 1.00 hr (0.50-1.75), 1.00 hr (0.75-1.75) and 8.00 hr (1.00-12.00), respectively. The mean±SD C_{max} (ng/mL) values in T1, T2 and R groups were 101.91±73.07, 51.67±14.81 and 18.89±7.89, respectively. The results for the extent of absorption, as determined by AUC_{0-t} (ng/mL) were 385.10±89.29, 349.63±64.41 and 371.50±56.25 in the in T1, T2 and R groups, respectively. Similarly, the results of $AUC_{0-\infty}$ were also comparable in all three arms. The mean±SD t½ (h) values were 15.43±5.81, 15.27±6.68 and 19.80±6.35 in T1, T2 and R groups, respectively.

Parameter (Unit)	Test product-T1 (n=51)	Test product-T2 (n=50)	Reference product-R (n=51)
C_{max} (ng/mL)	101.91±73.07	51.70±14.84	18.89±7.89
T_{max} (h)*	1.00 (0.50-1.75)	1.00 (0.75-1.75)	8.00 (1.00-12.00)
AUC_{0-t} (ng.h/mL)	385.19±89.31†	351.74±61.59	371.76±56.40†
$AUC_{0-\infty}$ (ng.h/mL)	392.85±90.56†	358.97±62.03	381.64±55.89†
t½ (h)	15.48±5.82†	15.40±6.58	19.83±6.32†

[Table/Fig-5]: Pharmacokinetic (PK) parameters of progesterone (baseline uncorrected data) in three arms.
Data presented as Mean±SD; *T_{max} is represented as median (min-max) value; †N=50.
Test 1: single 25 mg (1.119 mL) intramuscular injection of aqueous progesterone; Test 2: single 25 mg (1.119 mL) subcutaneous injection of aqueous progesterone; and Reference: single 25 mg (0.5 mL) intramuscular injection of oil-based progesterone formulation.
AUC_{0-t}, area under the curve from time 0 to t; $AUC_{0-\infty}$; area under the curve from time 0 extrapolated to infinite time; C_{max}, maximum plasma drug concentration; t½, plasma half-life; T_{max}, time to reach C_{max}

Baseline uncorrected data: The baseline uncorrected data were also in line with baseline corrected data [Table/Fig-5].

Relative bioavailability (baseline corrected data): The relative bioavailability analysis (i.e., geometric least squares means, ratio, 90% CI, and intrasubject CV) of any two of the three arms for baseline corrected data of progesterone is summarised in [Table/Fig-6].

T1 vs. R				
Parameters (Unit)	Geometric least squares means			90% Confidence interval
	Test product-T1 (N=51)	Reference product-R (N=51)	Ratio (T1/R)%	
lnC_{max}	80.73	17.65	457.3	399.80-523.05
lnAUC_{0-t}*	375.98	366.19	102.7	98.44-107.06
ln$AUC_{0-\infty}$*	383.49	376.07	102.0	97.96-106.15
T2 vs. R				
Parameters (Unit)	Geometric least squares means			90% Confidence interval
	Test product-T2 (N=50)	Reference product-R (N=51)	Ratio (T2/R)%	
lnC_{max}	49.17	17.65	278.5	243.31-318.84
lnAUC_{0-t}	343.76	366.19*	93.9	90.01-97.90
ln$AUC_{0-\infty}$	351.94	376.07*	93.6	89.90-97.42
T1 vs. T2				
Parameters (Unit)	Geometric least squares means			90% Confidence interval
	Test product-T1 (N=50)	Test product-T2 (N=50)	Ratio (T1/T2)%	
lnC_{max}	81.24	49.49	164.1	141.34-190.63
lnAUC_{0-t}	375.79^	343.68	109.3	104.44-114.48
ln$AUC_{0-\infty}$	383.36^	351.81	109.0	104.26-113.89

[Table/Fig-6]: Relative bioavailability results for progesterone (baseline corrected data).
*N=50 and ^N=49.
Test 1: single 25 mg (1.119 mL) intramuscular injection of aqueous progesterone; Test 2: single 25 mg (1.119 mL) subcutaneous injection of aqueous progesterone; and Reference: single 25 mg (0.5 mL) intramuscular injection of oil-based progesterone formulation.
AUC_{0-t}, area under the curve from time 0 to t; $AUC_{0-\infty}$; area under the curve from time 0 extrapolated to infinite time; C_{max}, maximum plasma drug concentration

The ratio and 90% CIs for the extent of absorption parameters (lnAUC_{0-t} and ln$AUC_{0-\infty}$) were within 80 to 125% for comparison of any two arms; while the same data for C_{max} were not within 80 to 125%.

DISCUSSION

The novel water soluble progesterone formulation administered s.c. or i.m. was developed to reduce the injection site pain and discomfort, which are usually associated with the oil-based progesterone formulation administered intramuscularly [10,11]. The current study was a cross-over, single dose, PK bioequivalence comparison study of aqueous-based (both s.c. and i.m. routes) vs oil-based progesterone (i.m. route) formulations. In this study, healthy subjects were included for bioequivalence comparison of these formulations, and there was no disease/condition were aimed to be treated in this study. This study compared the PK properties of newly developed water soluble progesterone formulation with the marketed oil-based progesterone formulation in healthy postmenopausal female subjects. The aqueous-based progesterone administered s.c. or i.m. was rapidly absorbed leading to almost 3-5 times higher and earlier peak serum progesterone concentration compared to oil-based progesterone formulation. However, the extent of absorption was found similar in all three groups indicating similar bioavailability.

The PK results of aqueous-based progesterone formulation from this study were in line with the results of innovator formulation published earlier [19]. In the single-dose, randomised, three-way cross-over comparative PK study by Sator M et al., aqueous-based progesterone administered via i.m. and s.c. routes was compared with an oil-based reference formulation of progesterone administered as i.m. route in 12 postmenopausal women. The single subcutaneous administration of 25 mg dose of aqueous based progesterone formulation demonstrated the mean±SD C_{max} at 57.84±13.55, AUC_{0-t} at 337.65±91.58 and $AUC_{0-\infty}$ at 349.17±91.10, which are comparable to 51.67±14.81, 349.63±64.41 and 357.42±63.81, respectively, with the current study. Further, the plasma half-life and the time to reach maximum plasma concentration was comparable for a single 25 mg s.c. administration of aqueous progesterone formulation for the study reported by Sator M et al., and the current study [Table/Fig-7] [19].

Parameters (unit)	Progesterone aqueous based formulation (single 25 mg subcutaneous administration) (Mean±SD)	
	Present study	Sator M et al., [19]
T_{max} (h)*	1.0 (0.75-1.75)	0.92 (0.5-2)
C_{max} (ng/mL)	51.67±14.81	57.84±13.55
AUC_{0-t} (ng.h/mL)	349.63±64.41	337.65±91.58
$AUC_{0-\infty}$ (ng.h/mL)	357.42±63.81	349.17±91.10
t½ (h)	15.27±6.68	13.06±7.08

[Table/Fig-7]: Comparison of present study results with the published study from the innovator.
*Data presented as mean (min-max).
Test 1: single 25 mg (1.119 mL) intramuscular injection of aqueous progesterone; Test 2: single 25 mg (1.119 mL) subcutaneous injection of aqueous progesterone; and Reference: single 25 mg (0.5 mL) intramuscular injection of oil-based progesterone formulation.
AUC_{0-t}, area under the curve from time 0 to t; $AUC_{0-\infty}$; area under the curve from time 0 extrapolated to infinite time; C_{max}, maximum plasma drug concentration; t½, plasma half-life; T_{max}, time to reach C_{max}

The results of this study were also comparable in terms of exposure parameters (C_{max} and AUC) to the dose normalised data (from 100 mg to 25 mg) from a study by Sator M et al., which compared the PK parameters of single 100 mg dose of either i.m. or s.c. administration of innovator aqueous-based progesterone formulation or i.m. oil-based formulation [19]. The cross-over design was adapted to minimise intrasubject variability. The PK analyses were performed as per the non compartment model, similar to as reported in the study by Sator M et al., [19]. The clinical utility of the aqueous-based progesterone formulation was evaluated in 800 women undergoing In-vitro Fertilisation (IVF) by Baker VL et al., who reported that aqueous-based progesterone formulation administered as s.c. injection was similarly effective in luteal phase support as compared with vaginal progesterone formulation [10]. In this study, all reported AEs were mild in nature and there were no clinically significant differences in the incidence of AEs between all three groups.

The current study demonstrated a bioequivalence among between the test (aqueous-based progesterone formulation administered via s.c. or i.m. routes) and reference (oil-based progesterone formulation administered via i.m. route) formulations. The availability of a bioequivalent progesterone formulation in an aqueous media will provide the patients a better well tolerated alternative for their luteal phase support in the ART treatment. Progesterone is administrated for luteal phase support for 8-12 weeks of gestation in ART [7]. Progesterone oil-based injection administered daily i.m. or vaginal progesterone are commonly used for luteal phase support in ART. Intramuscular progesterone oil-based injection is associated with pain at injection site, discomfort, and even sterile abscess formation and poor compliance [9,13]. In contrast to progesterone oil-based i.m. injections, aqueous progesterone injection is well tolerated. It also avoids the side-effects of vaginal progesterone such as vaginal irritation and discharge [10,12]. Aqueous progesterone injection also provides advantages like convenience of s.c. administration enabling ease of use. It is a novel, safe, effective and a patient friendly option for luteal phase support in women undergoing ART, where a patient requires daily administration of progesterone [20].

Limitation(s)

The study's limitations included open-label or observer-blinded study design. However, mitigation strategies for potential bias were applied and the analysis of the study samples were performed using validated softwares.

CONCLUSION(S)

In conclusion, the aqueous-based progesterone formulation administered s.c. or i.m. demonstrated similar bioavailability as the reference oil-based progesterone formulation administered i.m. Considering the advantages in terms of comfortable administration, and possibility of self-administration via the s.c. route, along with the better tolerability profile of the aqueous-based progesterone formulation, it could offer a convenient alternative to oil-based formulations for luteal phase support to patients undergoing ART treatment.

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Conflict of interest: Dr. Sonal and Dr. Alok are employees of Intas Pharmaceuticals Limited, Ahmedabad, India.

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