International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 10; Issue 06 (A); June 2021; Page No.24580-24584
DOI: http://dx.doi.org/10.24327/ijcar.2021.4879.24584

Research Article

PHARMACOKINETICS OF ALTRENOGEST AFTER ORAL DOSING IN HEALTHY NULLIPAROUS SOWS

Gonzalo López Rincón[1]., Elba M. Romero Tejeda[2]., Eligio R. Moreno-Gómez[3]., Leonel Avendaño Reyes[4] and Guillermo Oregel Ramírez[1]

[1]Department of Research and Development, Virbac México S.A de C.V. Zapopan, Mexico
[2]Department of Pharmacobiology, CUCEI-University of Guadalajara, Guadalajara, Mexico
[3]Department Animal Production, CUCBA-University of Guadalajara. Zapopan, Mexico
[4]Institute of Agriculture Science, Autonomous University of Baja California, Mexicali, Mexico

A R T I C L E I N F O

Article History:

Received 15th March, 2021
Received in revised form 7th April, 2021
Accepted 13th May, 2021
Published online 28th June, 2021

Key Words:

Altrenogest, Gilts, Pharmacokinetics, Oral administration.

A B S T R A C T

Background: The Altrenogest has been widely used to induce estrus synchronization in gilts, but there are no data on the pharmacokinetics profile as a method for synchronization on commercial pig farms. The purpose of this study was to determine the pharmacokinetic profile after 18 oral doses of 20 mg Altrenogest/gilts/day at an interval of 24 h. **Material and methods**: Blood samples from each gilt were collected before (0 h) and after drug administration at 20 different time points between 1–504 h. Plasma samples were collected, and drug plasma concentrations were determined by a validated high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) method. Non-compartmental pharmacokinetic analysis was performed using Kinetica® version 5.1 software. **Results:** After the first administration (D1), the pharmacokinetics parameters, including C_{max}, AUC_{tot} and $AUC_{last,}$ and V_{ss}, were statistically significantly lower that after multiple doses in gilts D9 and D18 ($p \leq 0.05$). Others parameters, T_{max} and $T_{1/2\lambda z}$, were similar to those observed after the final administration D18 ($p \geq 0.05$). **Conclusion:** The results revealed that Altrenogest plasma concentretion show significant accumulations over the time of treatment. The fluctuation might be caused by Altrenogest liposolubility according to different physiological stages and body weigh especially in nulliparous sows. However, further research is required in this area in order to determine Altrenogest pharmacokinetic parameters according to synchronization protocol on commercial pig farms.

INTRODUCTION

The practice of hormones is a tool used to induce puberty in gilts and to homogenize groups so that inseminations, births, and weaning are concentrated in a certain period, facilitating routine farm management through estrus synchronization (Martinat-Botté *et al*., 1990; Pereira de Souza *et al*., 2018; Wang *et al*., 2018). Altrenogest (allyltrenbolone, 17a-allyl-17b-hydroxy-estra-4,9,11-trien-3-one) is a synthetic steroid hormone an orally active progestogen, with progestogenic activity, belonging to the 19-nor-testosterone series, that postpones the onset of the follicular phase (EMEA, 2004, Machnik *et al*., 2007, VanLeeuwen *et al*., 2015).

Corresponding author:* **Gonzalo López Rincón
Department of Research and Development, Virbac México S.A de C.V. Zapopan, Mexico

Therefore, the use of Altrenogest has been a standard tool to synchronize the estrous cycle in mature sows and gilts (young female pigs) in commercial pig farms (Kraeling and Webel, 2015; Lopes *et al*., 2017) when fed for 18 days (20 mg/animal/day) it produces estrus, on average, 4 to 8 days after its removal from the feed (or 22 to 24 days after the first day of feeding) (Fernandez *et al*., 2005; Wang *et al*., 2018). Females for 18 days can be at any stage of the estrous cycle at the onset of feeding because Altrenogest is a progesterone-like compound, with a quiescent on the release of GnRH and the growth of follicles. In addition, Altrenogest is used widely to synchronize the estrous cycle or enable the occurrence of estrus to be predictable in groups of gilts, primiparous sows, mares, ewes, and cows (Kraeling and Webel, 2015). Reproductive efficiency would be enhanced if there were an effective method for synchronizing oestrus in replacement gilts (Johnson *et al*., 2016; Ziecik *et al*., 2020).

For many years, the use of Altrenogest for 18 days has been shown to induce the synchronization of gilts. Altrenogest is the

only product commercially available for controlling estrus and ovulation in swine females (De Rensis *et al*., 2016). In contrast, the plasma pharmacokinetic profile of Altrenogest as a commercial productin gilts has not been studied in veterinary research, and there are no relevant scientific studies of pharmacokinetic parameters on Altrenogest after orally administered to gilts under commercial production systems. Therefore, the purpose of this study is to describe the pharmacokinetic parameters of Altrenogest following oral administration in healthy gilts during 18 consecutive days when used to synchronization protocol in commercial pig farms.

MATERIALS AND METHODS

Animals

Eight clinically healthy PIC-Cam borough ®/PIC®337 nulliparous sows, Virbac-owned pubescent gilts, of 6 months old and weighing 130-160 kg (mean weight ± SD = 143 ± 13.7 kg) were enrolled in this study. All animals were considered healthy based on clinical history, physical examination, and hematology (complete blood count and serum chemistry analysis). After arrival, the gilts were housed in individual pens for the acclimation period and during sample collection under good hygienic conditions. Throughout the experiment, gilts were fed individually twice daily (8 am and 4 pm), receiving 3 kg of a standard sow ration per day (MPG® *ad libitum*). Water was available *ad libitum* through automatic pacifier-type drinkers.

The clinical parameters (attitude, appetite, depression, nasal and eye discharges, coughing, fever, and vital signs) were recorded twice daily throughout the study period. None of the gilts were treated with progestogens for one month. Before study enrolment. The Commission approved all procedures on Ethics and Animal Welfare of Virbac Mexico S.A de C.V according to the SOPVBMX-10-B-707. All animals were housed at the Animal Unit of Virbac Mexico S.A de C.V for the study duration.

Animal Procedures

The gilts were acclimated to the Animal Unit of Virbac Mexico S.A de C.V for 38 days before treatment with the test element Altrenogest to obtain a bodyweight of between 130 and 160 kg. Upon their arrival to the animal unit, the gilts were treated according to the SOP VBMX-7-L-140, handling, and inventory of animals. During that period, the test systems were trained and adapted to the procedure in which the test element Altrenogest would be administered, using apple juice and the dosage device included in the product. The clinical phase was executed in a single stage which consisted of the administration of the test element (Altrenogest) for 18 consecutive days; blood samples of about 7 mL each (Vacutainer® NH Sodium Heparin, Greiner Bio-One North America, Inc., Monroe, NC), were collected from the jugular vein just prior dosing and at 1, 3, 6, 9, 24, 192,193, 185, 198, 201, 216, 408, 409, 411, 414, 417, 432, 456, 408 and 504h post first treatment. Blood samples were stored at 8°C for 2 h, and plasma was separated after centrifugation at 2,500 g for 15 minutes, placed in 2.0 mL screw-top Cryogenic vials (Cryogenic Vials 2.0 mL, Sterile, Fisherbrand, Pittsburgh, PA, U.S.A.).

The plasma samples were aliquoted and stored at −80 °C until high-press liquid chromatography (HPLC MS/MS) analysis. The samples were analyzed within 30 days of freezing. Plasma concentrations of Altrenogest were measured by high-performance liquid chromatography coupled to a tandem mass spectrometer (HPLC-MS/MS), and the concentration-time data were analyzed by Kinetica, version 5.1 (Thermoelectron Crop, U.S.A).

Drug administration

Altrenogest oral solution treated gilts received their daily dosage of Altrenogest (20 mg, VIRBAGEST®, Virbac Mexico S.A de C.V) as a top dressing over their morning feed. Treatment lasted 18 days.

Sample analysis

The previously reported (HPLC-MS/MS) method was validated for accuracy, selectivity, linearity, precision, stability, and sensitivity. Quantitation was based on a calibration curve generated by spiking known concentrations (0.5000–70 ng/mL) of Altrenogest (T.L.C. Pharmaceutical; Standards) into water and Letrozone as an internal standard (U.P.S.) according to the European Medicines Agency 2011, (Guideline on bioanalytical method validation). The limit of quantitation L.O.Q. (determined as the lowest standard at which accuracy of at least 20% was routinely achieved) for the assay was 0.5000 ng/mL, and the limit of detection L.O.D. was 0.1667 ng/mL. Very briefly, 300 µl of plasma was added to 300 µl of methyl terbutyl ether/Hexane (HPLC grade; Tedia). The plasma sample was vortexed vigorously to mixfor 4 min and centrifuged at 16,000x g for 20 min at 4°C (centrifuge Hermle Z446K). The supernatant was passed through a 0.22 µm membrane before being injected into the UPLC-MS/ MS system (Prominence HPLC systems; HPLC, L.C. 20A, Shimadzu Corp., Kyoto, Japan, MS/MS ABSCIEX). UPLC separations were conducted on an Agilent Poroshell (120 EC-C_{18} 2.7 µm 3.0 x100 mm) at 40°C. The separation was performed with 0.1% formic acid (Reactive grade; C.T.R., U.S.A.) (mobile phase solvent A) and acetonitrile (solvent B) in 20:80 proportion. The flow rate was 0.7 mL/min, with a total run time of 1.5 minutes. The injection volume was 10 µL. The mass spectrometry analysis conditions were as follows: Electrospray ionization in positive mode; and multiple reaction monitoring (M.R.M.) and transitions of Altrenogest (311.3-227.2 UMAS) and Letrozole (286.2-217.0 UMAS).

Pharmacokinetic and statistical analysis

Non-compartmental pharmacokinetic parameters were evaluated using Kinetica® version 5.1 software (Thermoelectron Crop, U.S.A). The main pharmacokinetic parameters were presented as follows: (C_{max}): The maximum concentration of drug in plasma; (T_{max}): Peak plasma concentration-time; ($T_{1/2}$): Elimination half-life; (M.R.T.): Mean residence time; (AUC_{tot}): Total area under the curve; (AUC_{last}): Area under the plasma concentration-time curve from time zero to time of last measurable concentration; (AUC_{extra}): Area under the plasma concentration-time curve extrapolated from time t to infinity as a percentage of total AUC; (Cl): Clearance time; (λz) Terminal elimination rate constant; (Vss): Apparent volume of distribution at steady state; (Vz): Volume of distribution in the terminal state. C_{max} and T_{max} were determined directly from the experimental data of each animal. Statistical analysis was performed with a one-way ANOVA compared the means of each peak and trough concentration of three independent days in order to determine

whether there was statistical significance for the results. The Dunnett's Multiple Comparison Test was used to adjust for multiple comparisons. Data were analyzed using GraphPad Prism version 5.03 software (Inc., La Jolla, CA, U.S.A.). Significance was defined as ($p \leq 0.05$). The results were presented as mean ± (S.D.).

RESULTS

Adverse reactions were not detected in any of the animals following 18 oral administrations at a dose of 20 mg gilt^{-1} day^{-1}. All animals showed no clinical abnormalities during the period of the experiment. Plasma Altrenogest concentrations were measured for 8 gilts at D1, D9, and D18 after the treatment and determined by liquid chromatography/mass spectrometry analysis (Figure 1).

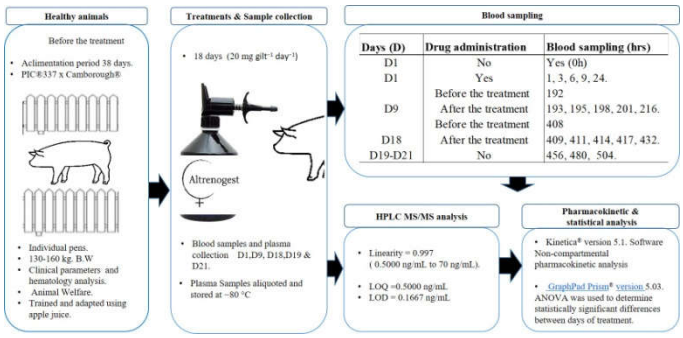

Figure 1 Experimental workflow under commercial pig farms. Description of the study development process, with the indications of workflow. The blood sampling table corresponds to the dosing and sampling regimen of blood before and after treatment with Altrenogest. The black arrows correspond to the workflow until the finalization of the results.

The mean ± S.D. plasma concentration *versus* time data after oral administration of 20 mg of Altrenogest in gilts are provided in (Table 1). On D1, the mean peak time (*Tmax*) was obtained at 3.75 ± 2.96 h with the mean concentration (C*max*) of 13.24 ± 6.14 ng/mL. $T_{1/2}$ and M.R.T. were 13.47 ± 9.48 h and 21.12 ± 14.15 h, respectively. AUC_{tot} was 238.29 ± 59.52, and AUC_{last} was 150.05± 49.57 ng h/mL. After 24 h, the mean plasma Altrenogest concentrations decreased time-dependent to 3.67 ng/mL± 0.9 (Table 1; Figure 2).

On D9, the mean peak time (T_{max}) was obtained at 2.13 ± 1.81 h with the mean concentration (C_{max}) of 45.50 ± 12.35 ng/mL. $T_{1/2}$ and M.R.T. were 10.46 ± 8.51 h and 15.89 ± 12.72 h, respectively. AUC_{tot} was 590.16± 192.02, and AUC_{last} was 464.30 ± 137.47 ng h/mL.

After 24 h, the mean plasma Altrenogest concentrations decreased time-dependent to 6.78 ng/mL (Table 1; Figure 2).

Finally, On D18, the mean peak time (T_{max}) was obtained at 5.13 ± 2.75 h with the mean concentration (C_{max}) of 30.71 ± 14.11 ng/mL. $T_{1/2}$ and M.R.T. were 15.36 ± 7.30 h and 23.93 ± 10.13hr, respectively. AUC_{tot} was 662.16 ± 228.17, and AUC_{last} was 426.41 ± 145.72 ng h/mL. After 24 h, the mean plasma altrenogest concentrations decreased time-dependent to 11.48 ng/mL. During prolonged treatment, accumulation of Altrenogest was observed in the plasma of gilts, showing an increasing trend as the administration continued. There was significant accumulation after 18 consecutive days of the administration, had statistically significant difference between D1-24 h (3.67 ng/mL), D9-24 h (6.78 ng/mL), and D18-24h (11.48 ng/mL) post-treatment ($p \leq 0.05$). Also, peak plasma concentrations occur after 1-2 hours post-treatment; there was a significant increment with a statistically significant difference between D1-1h, D9-1h, and D18-1h post-treatment ($p \leq 0.05$). There was a significant difference among peak concentrations ($p \leq 0.05$), the same as trough concentrations ($p \leq 0.05$; Figure 2).

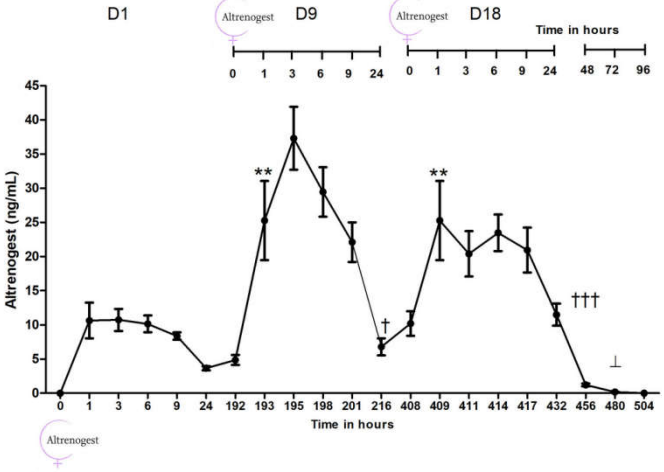

Figure 2 Plasma concentration of Altrenogest in healthy gilts following 18 oral administrations at a dose of 20 mg gilt^{-1} day^{-1}. Concentration–time diagrams are shown in detail within the first 24 h post-administration for day 1, day 9, and day 18. Data are expressed as mean ± SD, and significance between D1-24 h (3.67 ng/mL), D9-24 h (6.78 ng/mL) and D18-24 h (11.48 ng/mL) post-treatmentis defined as $p \leq 0.05$*, $p \leq 0.01$**. Significance between D1-1h, D9-1h and D18-1h post-treatment is defined as $p \leq 0.05$†, $p \leq 0.001$†††. Significance between D18, D19, D20, D21 and post- last treatment is defined as $p < 0.000$⊥.

Table 1 Pharmacokinetic parameters of Altrenogest after 18 oral administrations (20 mg gilt^{-1} day^{-1}) in healthy gilts (n = 8)

		Altrenogest treatment						
		D1		D9		D18		
Parameters	Units	Mean	(+/-SD)	Mean	(+/-SD)	Mean	(+/-SD)	P
C_{max}	ng/mL	13.24	6.14	45.50	12.35	30.71	14.11	0.001***
T_{max}	h	3.75	2.96	2.13	1.81	5.13	2.75	0.095
$T_{1/2\lambda z}$	h	13.47	9.48	10.46	8.51	15.36	7.30	0.438
MRT	mL/h	21.12	14.15	15.89	12.72	23.93	10.13	0.372
AUC_{tot}	ng/mL*h	238.29	59.52	590.16	192.02	662.16	228.17	0.001***
AUC_{last}	ng/mL*h	150.05	49.57	464.30	137.47	426.41	145.72	0.001***
AUC_{extra}	ng/mL*h	88.24	61.40	125.86	161.75	233.05	133.53	0.053
$\%AUC_{extra}$	%	35.68	19.82	17.86	18.18	34.15	13.49	0.064
CL	L/h	88.79	11.06	37.40	12.61	33.09	12.61	0.001***
λz	1/h	0.07	0.04	0.09	0.03	0.05	0.02	0.084
Vss	L/g	1.67	0.74	0.51	0.25	0.79	0.32	0.002**
Vz	L/g	1.52	0.73	0.49	0.25	0.70	0.30	0.004**

Data are presented as mean ± S.D. Significance between D1, D9, and D18 post-treatment is defined as $p \leq 0.05$*, $p \leq 0.01$** and $p \leq 0.001$***.

Statistical comparisons found that the C_{max}; AUC_{tot}, and AUC_{last}, were significantly higher following first administration on day D9 (3.43 fold, 2.47 fold, and 3.09 fold respectively) ($p \leq 0.05$). A similar result was also seen on D18 (2.13 fold, 2.77 fold, and 2.84 fold, respectively) ($p \leq 0.05$). (Table 1). In contrast, at steady state (*Vss*) and volume of distribution in the terminal state ($V_{Z.}$) exhibited a linear decrease after treatment on D9 (3.27 fold and 3.05 fold respectively) ($p \leq 0.05$). A similar result was also seen at the end of the experiment.

The data presented in Figure 2 indicated that the plasma concentrations of Altrenogest were significantly decreased after the last treatment (24 h=11.48; 48 h=1.22 ng/mL, 72 h=0.17 ng/mL and 96 h=0.0 ng/mL respectively) ($p \leq 0.0001$).

Our study also found that Altrenogest maintains the long-term effective blood concentration of the gilt ≥3.67 ng/mL, and it is enough to maintain the effect on estrus synchronization.

DISCUSSION

The administration of progestogens has proven to be highly effective in synchronizing estrus in cyclic sows and gilts (Dimitrov *et al*. 2010). Altrenogest is a potent synthetic progestin, is often used as an equine and swine veterinary pharmaceutical; it is widely used in the estrus synchronization of gilts, sows, and mares (Kraeling & Webel, 2015).

The pharmacokinetics of Altrenogest has been reported in horses administered every 24 hours for 5 days at 0.088 mg/kg (Ellis *et al*., 2019). In growing pigs was administered a single oral solution of experimental prototypes (Altrenogest) at1 mg/kg body weight (Li Y *et al*. 2020), and in gilts was administered daily at 20 mg $gilt^{-1}$ day^{-1} for 18 days of pharmaceutical prototypes (altrenogestsolution) (Ningbosansheng Pharmaceutical Co., Ltd, Zhejiang, China) mixed with 500 g of a commercial diet (Xiao *et al*., 2019). However, none administered as a commercial drug product according to synchronization protocols on commercial pig farms. Given this, a pharmacokinetic study was conducted on gilts to better guide reproductive clinical practice on commercial pig farms. This study showed that after the first administration of Altrenogest in healthy gilts, it was absorbed quickly after oral administration (T_{max} 3.75 h) with C_{max} of 13.24 ± 6.14 ng/mL, and the elimination half-lives were 13.47 ± 9.48 h. Previously, oral Altrenogest treatment at an oral dose of 44 µg/kg B.W. for 5 days was determined C_{max} of 35 ng/mL at 1 h after oral administration followed by rapid elimination and a decline to levels of 3 ng/mL by 24 h after administration in mares (Machnik *et al*., 2007). A recent study determined (T_{max} 4.25 h) with C_{max} of 16.00 ± 5.80 ng/mL and the elimination half-lives was 7.01 ± 3.13 h when given at an oral dose of 0.088 mg/kg B.W. in horses (Ellis *et al*., 2019). Pharmacokinetics studies of Altrenogest-pharmaceutical prototypes at 20 mg $gilt^{-1}$ day^{-1} in pigs report a (T_{max}1.96 h) with C_{max} of 66.16 ± 19.94 ng/mL and the elimination half-lives were 7.24 ± 0.98 h in gilts (Xiao *et al*., 2018). In another study, oral Altrenogest treatment with experimental prototypes after a single administration at 1 mg/kg B.W. was determined (T_{max} 1.16 h) with C_{max} of 227.59 ± 83.35 ng/mL, and the elimination half-lives were 3.63 ± 0.72 h in growing pigs (Li Y *et al*., 2020). Our results are very similar to results reported by Ellis *et al*., 2019 in horses. In contrast, a marked variability of pharmacokinetic parameters was observed. It is well recognized that the clinical response to drug administration varies widely between individuals and species. Among the many factors responsible for the variation in veterinary drug response, age and dose are relatively crucial due to impaired absorption, distribution, metabolism, and excretion of drugs. However, the plasma concentration of Altrenogest using commercial drug products according to synchronization protocols on commercial pig farms has not previously been reported.

This study was designed to describe the difference in the plasma kinetic profile between D1, D9, and D18 in daily oral administration when used in estrus synchronization dose regimens on commercial pig farms. Our results showed significant differences on C_{max}, AUC_{tot}, and AUC_{last} between day D1, D9, and D18 ($P \leq 0.001$) (Table:1). Most of Altrenogest was eliminated within 24 h with a low trough concentration before the subsequent dosing. In addition, the accumulation of a drug in the body was observed time-dependent. During prolonged treatment, accumulation in plasma is found in pigs (EMEA, 2004). These results showed that during repeated administrations, the plasma exposures C_{max}, $AUC_{tot,}$ and AUC_{last} of Altrenogest increase proportionally over the treatment time, showing on D9 a 3.43 fold increase in C_{max}, a 2.47 fold increase in $AUC_{\text{tot,}}$ and a 3.09 fold increase in AUC_{last} respectively. A similar result was also seen on D18, showing a significant difference after single and multiple doses ($p \leq 0.001$). In addition, the (V_{ss}) and ($V_{Z.}$) shown a decrease on D9 and D18 after the drug administration (Table 1) ($p \leq 0.05$). Lipophilic molecules are more likely to pass through lipid bilayers and, therefore, to leave the bloodstream and distribute to areas with high lipid density (adipose) and therefore have a higher volume of distribution (*Vd*) after first administration (Mansoor *et al*., 2020; Berezhkovskiy, 2004). The steady-state was reached between the 9^{th} and 18^{th} administrations and therefore had a lower *Vd*. The system was subjected to a constant-rate drug infusion into plasma so that all concentrations, which describe the drug distribution in the body, become unchanged significantly, and similar results have been observed for both D9 and D18.

The plasma kinetic profile data are limited in gilts according to synchronization protocols. The fluctuation might be caused Altrenogest acts by its liposolubility by penetrating the target cells where it binds to specific receptors; it could be stored in fat temporarily and reenter circulation when the drug is eliminated. It is a rational explanation, in this clinical trial the optimal bodyweight for a gilt at 1^{st} service was used (130 to150 kg), with ≥20 mm back-fat thickness (Lee *et al*., 2019). The age and weight at first mating are considered as critical factors in lifetime gilt production and these factors should be taken into account to establish the plasma kinetic profile of Altrenogest as an effective synchronization method to regulate the estrous in cycling gilts in commercial pig production systems.

CONCLUSION

In conclusion, the validated bio-analytical HPLC MS/MS technique with a convenient extraction procedure was used to quantify Altrenogest in gilts having a regression (r^2) value of 0.997. Our data suggest that Altrenogest is absorbed rapidly, and most of it disappears after 24 h of administration. There was significant accumulation after 18 consecutive days of administration. Therefore, C_{max}, AUC_{tot}, and AUC_{last} showed a

significant difference between D1, D9 and D18 ($p \leq 0.001$), indicating that during prolonged treatment accumulation in plasma is found in gilts. The fluctuation might be caused Altrenogest by liposolubility and physiological stage (age and weight at first mating); heavier gilts had a higher backfat. However, further research is required in this area in order to determine altrenogest pharmacokinetic parameters according to synchronization protocols on commercial pig farms.

Conflict of Interests

The authors declare that there is no conflict of interests.

Author Contributions

Gonzalo Lopez-Rincon, the corresponding author, contributed to methodology; project administration and was responsible for giving the final approval of the manuscript.

Guillermo Oregel-Ramirez. Contributed to methodology and follow-up clinical trial, evaluation and interpretation pharmacokinetics, and original draft writing.

Elba M.Romero-Tejeda. Contributed to review the assays for Altrenogest using HPLC-MS/ MS and results evaluation and interpretation.

EligioR Moreno-Gomez. Contributed to follow-up clinical trialand results from evaluation and interpretation.

Leonel Avendaño-Reyes. contributed to results evaluation and interpretation. All authors in this study declare that they have read and approved thefinal manuscript.

Acknowledgments

This study was supported by Virbac México S.A de C.V. We sincerely thank Dr. José Manuel Moreno Monroy for his assistance in the English language revision of this work.

References

1. Berezhkovskiy LM. Volume of distribution at steady state for a linear pharmacokinetic system with peripheral elimination. J Pharm Sci. 2004 Jun; 93(6):1628-40. doi: 10.1002/jps.20073. PMID: 15124219.
2. De Rensis F, Kirkwood RN. Control of estrus and ovulation: Fertility to timed insemination of gilts and sows. Theriogenology. 2016 Oct 1;86(6):1460-1466. doi: 10.1016/j.theriogenology.2016.04.089. Epub 2016 May 14. PMID: 27287469.
3. Dimitrov, Stanimir & Bonev, Georgi&Taseva, H.. (2010). Synchronization of estrous in gilts with Altrenogest. Agricultural Science and Technogy. 2. 3-5.
4. Ellis KE, Council-Troche RM, Von Dollen KA, Beachler TM, Bailey CS, Davis JL, Lyle SK. Pharmacokinetics of Intrarectal Altrenogest in Horses. J Equine Vet Sci. 2019 Jan;72:41-46. doi: 10.1016/j.jevs.2018.10.001. Epub 2018 Oct 10. PMID: 30929782.
5. EMEA, 2004. Committee for Medicinal Products for Veterinary Use Altrenogest. EMEA/MRL/904/04-FINAL.
6. Fernandez, L., Diez, C., Ordonez, J.M., Carbajo, M., 2005. Reproductive performance in primiparous sows after post-weaning treatment with a progestagen. J. Swine Health Prod. 13, 28–30.
7. Johnson JS, Martin KL, Pohler KG, Stewart KR. Effects of rapid temperature fluctuations prior to breeding on reproductive efficiency in replacement gilts. *J Therm Biol*. 2016 Oct; 61:29-37. doi: 10.1016/j.jtherbio. 2016.08.003. Epub 2016 Aug 19. PMID: 27712657.
8. Kraeling, R.R., Webel, S.K. Current strategies for reproductive management of gilts and sows in North America. *J. Anim. Sci. Biotechnol*. 6, 3 2015. https://doi.org/10.1186/2049-1891-6-3.
9. Lee, S., Hosseindoust, A., Choi, Y., Kim, M., Kim, K., Lee, J., Kim, Y., &Chae, B. (2019). Age and weight at first mating affects plasma leptin concentration but no effects on reproductive performance of gilts. *Journal of animal science and technology*, 61(5), 285–293. https://doi.org/10.5187/jast.2019.61.5.285.
10. Lopes, T., Bolarín, A., Martínez, E., Roca, J., 2017. Altrenogest treatment before weaning improves litter size in sows. Reprod. Domest. Anim. 52, 75–77.
11. Machnik, M., Hegger, I., Kietzmann, M., Thevis, M., Guddat, S., &Schanzer, W. (2007). Pharmacokinetics of Altrenogest in horses. [Journal Article; Research Support, Non-U.S. Gov't; Validation Studies]. *Journal of Veterinary Pharmacology Therapeutics*, 30(1), 86– 90.
12. Mansoor A, Mahabadi N. Volume of Distribution. [Updated 2020 Jul 27]. In: StatPearls [Internet]. Treasure Island (F.L.): StatPearls Publishing; 2021 Jan-. Available from: https://www.ncbi.nlm. nih.gov/books/NBK545280/.
13. Martinat-Botté, F.O., Bariteau, F., Forgerit, Y., Macar, C., Moreau, A., Terqui, M., Signoret, J.P. Control of oestrus in gilts II. Synchronization of oestrus with a progestagen, Altrenogest (Regumate): effect on fertility and litter size. Anim. Reprod. Sci. 1990; 22, 227–233.
14. Pereira de Souza Labêta C, Da Silva de Vasconcelos B, Bourg de Mello M.R. Induction of puberty and synchronization of estrus in gilts with eCG and GnRH. R. Bras. Zootec; 2018 47:e20170342, 2018.
15. Van Leeuwen JJ, Martens MR, Jourquin J, Driancourt MA, Wagner A, Kemp B, Soede NM. Follicle size and reproductive hormone profiles during a post-weaning altrenogest treatment in primiparous sows. ReprodFertil Dev. 2015 Jan;27(2):304-12. doi: 10.1071/RD13149. PMID: 24192203.
16. VBMX-10-B-707.2020. Commission on ethics and animal welfare.
17. VBMX-7-L-140.2020. Manejo e inventarios de animales.
18. Wang Z, Liu BS, Wang XY, Wei QH, Tian H, Wang LQ. Effects of altrenogest on reproductive performance of gilts and sows: A meta-analysis. AnimReprod Sci. 2018 Oct;197:10-21. doi: 10.1016/j.anireprosci. 2018.08.035. Epub 2018 Aug 30. PMID: 30197055.
19. Xiao H, Sun P, Sun F, Qiu J, Wang J, Wang J, Lin Y, Gong X, Zhang L, Zhang S, Cao X. Pharmacokinetics of Altrenogest in gilts. *J Vet Pharmacol Ther*. 2019 Nov;42(6):660-664. doi: 10.1111/jvp.12755. Epub 2019 Jun 20. PMID: 31222770.
20. Li Y, Yang H, Xia L, Wang S, Bu S. Comparative pharmacokinetic study of two kinds of altrenogest oral solutions for sows. AnimBiotechnol. 2020 Mar 17:1-7. doi: 10.1080/10495398.2020.1723608. Epub ahead of print. PMID: 32180500.
21. Ziecik AJ, Drzewiecka K, Gromadzka-Hliwa K, Klos J, Witek P, Knapczyk-Stwora K, Gajewski Z, Kaczmarek MM. Altrenogest affects the development and endocrine milieu of ovarian follicles in prepubertal and mature gilts†. BiolReprod. 2020 Oct 29;103(5):1069-1084. doi: 10.1093/biolre/ioaa136. PMID: 32744329.