Macrothink Institute™

Journal of Agricultural Studies
ISSN 2166-0379
2022, Vol. 10, No. 4

Fluazuron Influences the *in vitro* Production Embryos of Wagyu Cow

Vinicius Eduardo Gargaro Silva, Fábio Luiz Bim Cavalieri, Márcia Aparecida Andreazzi (Corresponding author), José Eduardo Gonçalves, José Maurício Gonçalves dos Santos, Danieli Aparecida Bóbbo Moreski, Laura Paulino Mardigan, Tales da Silveira Faria

Cesumar University/ UNICESUMAR, Postgraduate Program in Clean Technologies and Veterinary Medicine Course, Avenue Guedner, Postal Code 87050-390 Maringá, PR, Brazil.
Email: marcia.andreazzi@unicesumar.edu.br

Received: June 28, 2022 Accepted: August 5, 2022 Published: August 7, 2022

doi:10.5296/jas.v10i4.20039 URL: https://doi.org/10.5296/jas.v10i4.20039

Abstract

Imported breeds raised in tropical countries demand greater use of chemical control of ectoparasites, however, this practice can compromise the results of biotechnologies of reproduction. Thus, the objective of this study was to evaluate the presence of chemical residues of tick or their metabolites in the blood and in the follicular fluid and to verify the in vitro embryo production (IVEP) of Wagyu cows, raised in a tropical country, submitted to fluazuron based tick treatment. Twenty adult Wagyu bovine females were used, donors of oocytes, divided into 2 groups: G1 - animals that were not submitted to tick control and G2, animals that were submitted to chemical control of ticks with fluazuron based product (2.5 mg kg-1 of body weight). After application (D0), all cows were submitted to estrous synchronization and, in four moments (D12, D33, D54 and D75) the aspirations of the follicular fluid from the dominant follicles were performed, the oocytes were collected for IVEP and were collected blood samples for extraction and analysis of the presence of chemical residues, using GC-MS. Plasma residues of fluazuron were detected up to 54 days after application of the tick, but no residues were detected in the follicular fluid. Group 1 had a higher number of total and viable oocytes ($p<0.0001$), however, the viability rate and the blastocyst rate was lower ($p<0.0001$), showing that the use of ticks compromised IVEP.

Keywords: biotechniques of reproduction, cattle tick pesticide, follicular fluid, chemical waste

1. Introduction

1.1 Cattle Farming in Brazil

In order to maintain good production rates in beef cattle herds, several factors must be taken into account, including sanitary management, as infectious diseases or the presence of endo

and/or ecotparasites can reduce the development rates of the herd (BERGE and VERTENTEN, 2017).

In tropical countries, an ectoparasite of economic importance is the tick *Rhipicephalus* (*Boophilus*) *microplus* (CHEFER & SOUZA, 2016), with the main form of control being chemical, based on the use of products called ticks. This problem is aggravated, mainly, with the introduction of imported breeds, which are more susceptible to ticks and, therefore, depend on more intensive control (MAPHOLI et al., 2014).

The Wagyu cattle breed, of Japanese origin, was introduced in tropical countries like Brazil, in order to expand the list of breeds raised and offer a product with differentiated quality, since its meat is recognized worldwide for its tenderness, juiciness and flavor, characteristics resulting from the carcass with high levels of marbling and the high amount of unsaturated fat (TANINAKA et al., 2015). Therefore, fostering this creation brings economic advantages to countries. However, as pointed out, imported breeds such as Wagyu, are more susceptible to ticks, a fact that demands a more intense control of ectoparasites.

Added to this fact, it is pointed out that for the maintenance or increase in the productive indexes of the bovine productive chain, it is necessary the use of biotechniques of reproduction, such as the *in vitro* production of embryos (IVPE), which allows the dissemination of high quality embryos and plays an important role in the genetic improvement of herds (GUSMÃO et al., 2017).

1.2 Research Justification and Objectives

Thus, considering that, there is a lack of research linking the use of chemical control of ectoparasites with the performance of IVPE cows and, in order to relate these points, the objective of this work was to evaluate the presence of chemical residues of fluazuron-based ticks in the blood and fluid. follicular and to verify the quality of the IVPE of Wagyu cows raised in a tropical country, submitted to the treatment with ticks..

2. Method

2.1 Ethical Animal Experimentation and Local

The procedures used in this research were approved by the Ethics Committee on the Use of Animals at the University Cesumar/ Unicesumar, under protocol No. 07/2020. The experiment was carried out at the Biotechnology Center/ Biotec, at farm school, Cesumar University / Unicesumar, Maringá, Paraná, Brazil (23°25'S, 51°57'W and altitude of 550 meters), in Southern Brazil, in 2020.

2.2 Management, Estrus Synchronization Protocol and Experimental Groups

Twenty adult Wagyu bovine females were used, donors of oocytes, aged between 12 and 24 months and with an average weight of 510.25 kg. The animals were in perfect body condition, with a body score between 3 and 3.5 and with excellent health and reproductive conditions. The animals were kept in paddocks of brachiaria (*Brachiaria brizantha* cv MG-5), with mineral supplementation and water ad libitum, being submitted to the hygienic management adopted in the property.

The animals were distributed in 2 experimental groups: Group 1 (G1) - animals that were not submitted to ectoparasite control and Group 2 (G2), animals that were submitted to control. Thus, on day zero of the experiment (D0), the G2 cows were subjected to carriage control with fluazuron based product (Tackzuron Pour On® - Zoetis), with a dosage of 2.5mg kg^{-1} of body weight , obtained with the application of 5.0 mL/50 kg of body weight, as established in the product insert. The total dose was administered in two bands, on both sides of the dorsal line, from the neck to the croup, with the product applicator. The grace period informed by the manufacturer for cattle for slaughter is 45 days after the last application (ZOETIS, 2022).

Afterwards, all cows were submitted to estrous synchronization, in four moments during the experiment, with intervals of 21 days. For synchronization, an auricular implant containing 5.0 mg of Norgestomet (progestin) (Crestar® - Lab. Intervet Ltda) and 2.0 mg of estradiol benzoate (EB) (Estrogin® - Lab. Farmavet Ltda.) was used intramuscularly. On days D7, D28, D59 and D71, the ear implant was removed and 2.0mL Prostaglandin F2α (PGF2α) (Ciosin® - Lab. Coopers Ltda) was applied, intramuscularly.

On days D12, D33, D54 and D75, aspirations of the follicular fluid from the dominant follicles were performed to analyze the presence of chemical residues. The follicular fluid was stored in microtubes and stored at -20ºC for further analysis. At the same time, the other follicles were aspirated to obtain the oocytes (Figure 1).

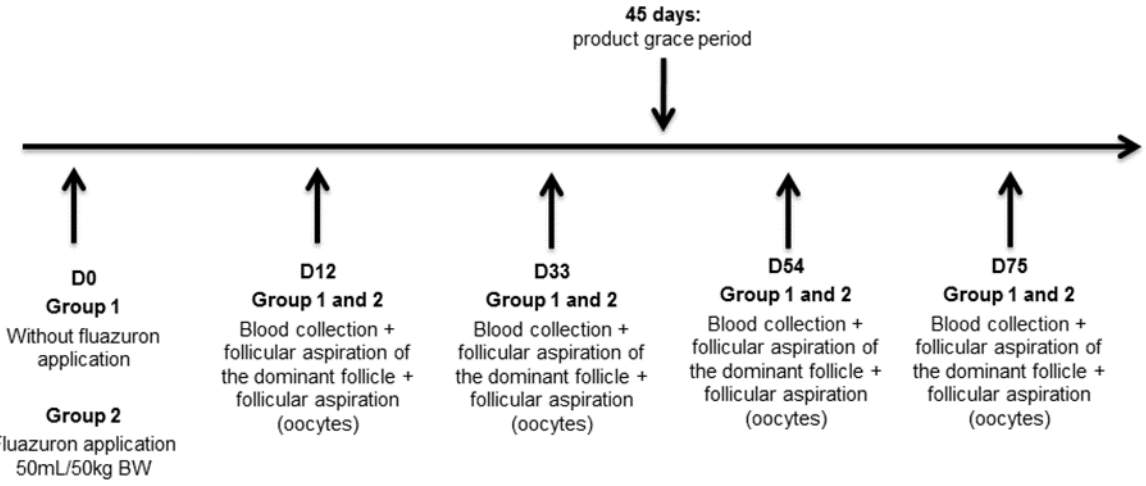

Figure 1. Experimental protocol

The aspiration of the follicular fluid from the dominant follicle was performed using an ALOKA ultrasound device type SSD500 with a 5 MHz convex sector probe adapted to a needle system (18G) and coupled to the vacuum system (pump) with the follicular aspiration corresponding to approximately 13 to 15 mL of water per minute. The aspirated follicular fluid was collected and transferred to microtubes and sent for analysis in the laboratory.

The aspiration of the other follicles to obtain the oocytes was also performed using an ALOKA ultrasound device type SSD500 with a 5 MHz convex sector probe adapted to a needle system (18G) and coupled to the vacuum system (pump). The vacuum pressure was obtained by means of a pump (Cook V-MAR 5000®), adjusted between 38 and 45mmHg,

allowing a flow of 12mL of half a minute. The oocytes were aspirated with a solution containing 2.0% Bovine Fetal Serum (BFS) (Nutricell®, Campinas, SP, Brazil), 25UI/mL of sodium heparin and 98.0% of Phosphate Buffer (PBS) (Nutricell®, Campinas, SP, Brazil).

At the time of follicular aspiration, low epidural anesthesia was performed with 5mL of 2% lidocaine (Pearson®, São Paulo/Brazil) to reduce peristaltic movements and discomfort in the animal. After lidocaine administration, the animal had the vulva washed and cleaned with paper towels. Then, the transducer was inserted to the bottom of the vaginal sac, with the manipulation of the ovaries through the rectum, seeking the best visualization of them on the ultrasound screen. The follicles were positioned on the puncture line indicated on the ultrasound screen and aspirated, according to the objective of the study. After the end of the aspiration, the vacuum system was cleaned with the means of receiving the oocytes and the used needles were discarded.

For the washing and selection of oocytes, the aspirated material was transferred to the embryo collection filter and washed with the same medium used for aspiration. The follicular liquid being separated into 50mL flasks and immediately afterwards, transferred to microtubes.

The remaining sediments in the filter were transferred to a Petri dish and the search, counting and classification of the oocytes as viable and non-viable was carried out. Oocytes that presented the presence of homogeneous cumulus and ooplasm were considered viable (grade I, II and III) and those that were nude or pycnotic, heterogeneous and with apoptotic vesicles were unviable (grade IV) (DE LOOS et al., 1989).

In vitro maturation (IVM) was performed in TCM199 with Earles salts (Gibco®), glutamine (Sigma® cod.:G8540) and $NaHCO_3$ (Mallinckrodt®), supplemented with 10% SFB (Cultilab®), 22µg/mL pyruvate (Biochemical® cod.:44094), 50 µg gentamicin (Sigma® cod.: G1272), 0.5 µg FSH/mL (Bioniche®), 50 µg LH/mL (Bioniche®) and 1 µg estradiol/mL (Sigma® cod.:E2758), kept in an oven at 39ºC, 5% CO_2 in air with maximum humidity, for 22 to 24 hours. The oocytes were placed in 90 µL microdroplets of maturation medium covered with mineral oil.

In vitro fertilization (IVF) was performed in 100 µL of TALP medium supplemented with 10µg/mL of heparin (Sigma cod.: H3149), 22µl/mL of pyruvate (Biochemical® cod.:44094), 50µg/mL of gentamicin (Sigma® cod:G1272), bovine serum albumin-BSA (without fatty acids) (Sigma® cod.:A3311), 2µM penicillin PHE solution (Sigma® cod.:P4875), 1µM hypotaurine (Sigma® cod.:H1384) and 0.25 µM of epinephrine (Sigma® cod.:E4250). The sexed semen of Wagyu bull was thawed in a water bath at 36ºC. For selection of mobile sperm and removal of thinners and seminal plasma, centrifugation in Percoll gradient (90 and 45%) was performed for 4 min at 4 rpm. $1x10^{-6}$ sperm/mL were used, and the oocytes were transferred to the microdroplets (20 oocytes/drop), where they remained for 18 hours, at 39ºC, in an atmosphere with 5% CO_2 in air.

After fertilization, the zygotes were cultured *in vitro* (CIV), in SOF medium (Synthetic Oviduct Fluid) supplemented with SFB (Nutricell®, Brazil), with granulosa cells monolayer.

Cultivation was carried out for 18 hours post-insemination, in an incubator, with a gaseous atmosphere containing 20% CO_2 in air, with maximum humidity.

Seven days after fertilization, the embryos were evaluated and packed in 0.25 mL straws to be later innovated in the recipients.

For the analysis of the presence of chemical residues of ticks in the blood, blood samples were taken on the same days as the collection of follicular fluid and oocytes, that is, days D12, D33, D54 and D75 after the application of the ticks, to elaborate the curve decay of the product's active ingredient and its metabolites. Samples of 10mL of blood were collected, by puncture of the jugular vein, in heparinized tubes, and were immediately subjected to centrifugation at 3500 rpm for 10 minutes. The plasma was separated, packed in microtubes, in duplicate of 1 mL each, and stored at -20ºC for further analysis.

For gas chromatography extraction and analysis, plasma samples were prepared by adding 10g of blood sample in a falcon tube, containing 250mg of magnesium sulfate, 250g of sodium chloride and 5mL of acetonitrile. The samples were shaken vigorously for 1 minute, followed by decanting by centrifugation (5.000 rpm) for 2 min. After phase separation, the top layer obtained from the mixture was transferred to QuEChERS cartridge, containing 50mg of $MgSO_4$, 15mg of PSA and 30mg of C18, to separate the desired analytes from the matrix, stirring vigorously for 1 minute and decanting by centrifugation at 5000 rpm for 2 min. For the analysis of the follicular liquid, the quantities were reduced, and the samples were prepared by adding 1g of sample of follicular liquid in a falcon tube, containing 125mg of magnesium sulfate, 125mg of sodium chloride and 3mL of acetonitrile, followed by stirring and decanting by centrifugation in the same way as plasma and, after phase separation, the upper layer obtained was transferred to QuEChERS cartridge, containing 1g of $MgSO_4$, 15mg of PSA and 7.5mg of C18, followed by agitation and mixing decanting. The separated upper layer, from both the plasma samples and the follicular liquid samples, was filtered and injected directly into the GC-MS. All extractions were performed in duplicate.

After preparing the samples, they were inserted into a gas chromatograph (model Agilent 7890B) with automatic injector (CTC PAL Control), coupled to a mass spectrometer (Model Agilent 5977A MSD), equipped with an HP-5MS UI Agilent column with 5% phenyl methyl siloxane phase (30.0m x 250µm di x 0.25µm film thickness). For the proper separation of the analytes in the GC-MS system, 2 µL of each extracted sample was injected into the column using the Split injection mode in a 1:50 ratio, in the following oven conditions: initial temperature of 70°C maintained by 2,5 min, then ramp from 15°C min^{-1} to 175°C maintained for 13 min, and ramp from 20°C min^{-1} to 290°C and maintained for 15 min.

The other conditions of the analysis method were: injection volume of 1.0µL, flow of carrier gas (He, purity 99.99999%) equal to 1.2 mL min^{-1}, ionization by electronic impact of 70 eV, ionization source temperature of 230°C, quadrupole of 150°C, transfer line of 280°C and injector of 250°C. Data acquisition was performed using the MassHunter software and qualitative analysis of mass spectra by the NIST 11 library. The quantification of the veterinary drug was performed using a pre-established calibration curve with a minimum level of 0.5 and a maximum of 8.0µg/mL and also by the presence of the internal standard of octadecane.

2.3 Statistics and Data Analysis

The data were tabulated and the statistical analyzes of the variables were performed using the PROC NPAR1WAY procedure of the statistical program SAS (2000), version 8.01, the means of plasma concentration and follicular fluid being analyzed by the Kruskal-Wallis test; for the analysis of the OPU efficiency, viability rate (percentage of viable oocytes in relation to the total oocytes) and of the blastocyst rate (percentage of embryos in relation to the total viable oocytes), the Poisson distribution and function of identity link.

3. Results

The result of the analysis of fluazuron in the blood, as expected, differed between the groups ($p<0.0001$) at 12, 33 and 54 days after application of the product, however, the drug was not detected on day 75 (Table 1).

Table 1. Plasma concentration of fluazuron in Wagyu donor cows subjected to a dose of 2.5 mg kg^{-1} of body weight of commercial formulation pour on fluazuron as a function of the days after application (µg mL^{-1})

Days after fluazuron application	G1 – control (n=10) (µg mL^{-1})	G2 – fluazuron (n=10) (µg mL^{-1})	p value
D12	0,0	3,80	<0,0001
D33	0,0	2,19	<0,0001
D54	0,0	0,85	<0,0001
D75	0,0	undetectable	

G1: group of animals that were not submitted to tick treatment; G2: group of animals that were subjected to treatment with ticks.

Regarding the efficiency of the Ovum pick-up (OPU), differences were observed between the groups in all variables studied ($p<0.0001$) (Table 2).

Table 2. Average efficiency of four sessions of *ovum pick-up* (OPU) and *in vitro* embryo production in Wagyu donor cows subjected to a dose of 2.5 mg kg^{-1} of body weight of commercial formulation pour on de fluazuron

Variables	G1- control (n=10)	G2- fluazuron (n=10)	p value
Total oocytes (M±SE)	11,8 ± 0,54	15,1 ± 0,61	<0,0001
Number of viable oocytes (M±SE)	8,77 ± 0,47	10,47 ± 0,51	<0,0001
Viability rate (%)	74,59 ± 1,36	68,68 ± 1,31	<0,0001
Number of non-viable oocytes (M±SE)	3,27 ± 0,28	4,6 ± 0,34	<0,0001
Number of embryos (M±SE)	3,05 ± 0,7	2,65 ± 0,6	<0,0001
Blastocyst rate (%)	34,73 ± 0,93	24,54 ± 0,78	<0,0001

G1: group of animals that were not submitted to tick treatment; G2: group of animals that were subjected to treatment with ticks. Viability rate: percentage of viable oocytes in relation to the total oocytes; Blastocyst rate: percentage of embryos in relation to the total viable oocytes; M±SE: mean and more or less standard error of the mean.

4. Discussion

The data on the analysis of fluazuron residue in blood are interesting because as pointed out in the product package insert, the grace period informed by the manufacturer for cattle for slaughter is 45 days after application (ZOETIS, 2022), therefore, it was expected that the same not be detected in the samples from the D54 collection. The decrease in serum levels was 42.37%, 77.63% and 100% in relation to the levels of fluzuron in D12.

In order to study the pharmacokinetic characteristics of fluazuron in cattle, Ferreira et al. (2019) evaluated and validated a method of liquid chromatography ultraviolet detection (LC-UV). The authors stated that fluazuron, administered topically to animals, with a dosage of 2.5mg kg-1, reached the systemic circulation and was absorbed (T max=48 Hs), remaining quantifiable in plasma levels for up to 14 days after treatment. This information differs from the findings of this study, since the drug was detected within 54 days after its application. Perhaps, this difference can be attributed to the sensitivity of the technique used, since, in this study, GC-EM was used, which is more sensitive than LC-UV.

The Committee for Medicinal Products for Veterinary Use, of the European Medicines Agency (EMA), published in 2017, a regulation establishing Maximum Residue Limits (MRL) for fluazuron in fin fish, valid throughout the European Union (EMA, 2017). In this document, the researchers stated that a highly specific method for analysis of fluazuron is the method of Liquid Chromatography Coupled to Mass Spectrometry (CL-MS), which, in fact, is more sensitive than the one employed in our study; however, in general, many different techniques are used in publications on fluazuron dosage.

Some studies, conducted with cattle of different breeds, and which applied doses of fluazuron, administered topically (*pour-on*), which varied between 1.25 to 2.5 mg kg^{-1} of body weight, reported values of residues of the drug in the plasma from 0.002 to 0.001 μg/mL, after 42 and 84 days, respectively (THOMAS et al., 1992; SWINDALE et al., 1993), revealing values well below those found in this research.

Brazil is one of the world's largest consumers of agrochemicals (HERMIDA et al., 2015). For this reason, several agencies are involved in determining the Maximum Residue Limits (MRL) for agricultural products, such as Codex Alimentarius (CODEX), the European Medicines Evaluation Agency (EMEA) and the National Health Surveillance Agency (ANVISA). Despite the high degree of asymmetry and subjectivity present in the decision criteria adopted by the countries and by the expert committees, it is observed that the MRL of fluazuron in cattle presented by the agencies are similar, with 7.000 μg/kg in fat, 200 μg/kg in muscle, 500 μg/kg in liver and kidney and 200 μg/kg in milk, however, the documents do not mention plasma levels.

Based on the technique and the calibration curve used in this study, it was not possible to detect chemical residues of fluazuron in the follicular fluid. It is pointed out that, perhaps, using an even more sensitive calibration curve, or if it was possible to obtain a larger volume of follicular fluid or employ a more effective follicular fluid pre concentration step, or use more sensitive analytical methods such as CL-MS perhaps the same could have been detected, despite the good sensitivity of the curve used in this study.

Bovine follicular fluid (FF) is an exudate from blood plasma with different protein factors, glycoproteins, glycosaminoglycan's, steroids and many other metabolites synthesized by cells in the follicular wall (GORDON, 2003). The composition of the follicular fluid is similar to that of serum with respect to low molecular weight components and most electrolytes (SHALGI et al., 1972). However, some components may vary, as the basal lamina of the follicle can act as a barrier for the diffusion of substances, such as proteins greater than 100 KDa (CLARKE et al., 2006), thus, another hypothesis is that the basal lamina has prevented the transfer of the fluazuron residue to the follicular fluid.

The importance of some FF constituents in acquiring oocyte development competence has already been reported. Steroids, such as estradiol and progesterone, are related to follicle growth and atresia, also having an effect on oocytes. In fact, the FF has a variety of functions related to the oocyte including maintaining meiotic arrest, protection against proteolysis, extrusion during ovulation and protection against adverse blood influences (SOHEL & CINAR, 2014) and, therefore, based on their importance physiological and the fact that residues of veterinary drugs can be found in various tissues (XIN-LUN et al., 2013; ZHANG et al., 2013), this research was conducted, however, without success in detection.

Regarding the efficiency of the OPU, it was possible to observe that, despite the greater number of total and viable oocytes, the viability rate, the number of embryos and the blastocyst rate in the group of donors that were treated with ticks were lower ($p<0.0001$). Based on these results, it appears that, in fact, as the number of follicles is mainly determined by genetic factors (BLONDIN et al., 2012), there was no effect of treatment on this variable, and however, when the structures were subjected in an in vitro culture system, the effect appeared. So, even if no fluazuron residue was detected in the follicular fluid, but considering the circulating levels in the blood and the negative effects obtained in the in vitro embryo production, it is believed that using an even more sensitive calibration curve it is possible to detect it and better elucidate these results.

However, it is reported that despite the significant effects found, the values obtained in this study are similar to those reported in other studies carried out with Wagyu donor cows. In a study whose objective was to investigate the follicular synchronization using estradiol benzoate, with the presence of the corpus luteum, in bovine female donors of Wagyu oocytes, in in vitro embryo production, Zamai et al. (2021) observed total oocyte values between 10.50 and 12.83, viability rate around 74%, total embryos produced between 2.40 to 2.85 and blastocyst rate between 19.16 to 26.44%.

However, it is considered that the number of total and viable oocytes obtained in the studies Zamai et al. (2021), regardless of treatment, was lower than those reported in the literature for other races (SARTORI et al., 2016), showing an inferiority in this aspect for Wagyu donors. However, despite this finding, the other reproductive advantages of Wagyu females stand out, such as sexual precocity, ease of delivery, maternal ability and longevity.

Studies in the scientific literature that assess follicular fluid, OPU and IVEP in animals submitted to tick treatment are scarce. However, research conducted by International Program On Chemical Safety (1997), with rats, evaluated the reproductive toxicity of fluazuron at

dietary concentrations of 0, 100, 1,500 or 20,000 mg kg^{-1} and pointed out that fluazuron did not cause adverse effects on function reproductive health of animals (IPCS, 1997).

Despite these results, Cabry et al. (2020) stated that exposure to pesticides can cause generalized oxidative stress, with high production of free radicals and impacts on the activity of the enzymes superoxide dismutase, catalase and glutathione peroxidase, reductase and transferase. Thus, it is assumed that these impacts may explain, in part, the results obtained in our study. Perhaps, the evaluation of oocytes through more in depth analyzes, such as metabolomics, can prove the effects of these compounds on the reproductive aspects of oocyte donor cows, since, the evaluation of recovered oocytes, fertilization and embryo cleavage directly reflect on the percentage of embryonic losses (AL-HUSSAINI et al., 2018), impacting the production chain.

5. Conclusion

Based on the results obtained, we conclude that it was possible to detect fluazuron residues in the plasma of Wagyu donor cows within 54 days after the application of a commercial tick pour fluazuron, however, fluazuron residues were not detected in the follicular fluid.

Regarding the efficiency of the *ovum pick-up*, despite the animals treated with ticks present a higher number of total and viable oocytes, the viability rate and the blastocyst rate were lower, demonstrating that the treatment negatively affected IVEP.

Thus, in order to better elucidate this effect, it is suggested that more research be conducted in order to establish a safer and more appropriate control protocol for ticks in cows of imported breeds, raised in a tropical country, donors of oocytes for IVEP.

Acknowledgments

The authors would like to thank the Cesumar Institute of Science, Technology and Innovation (ICETI/Brazil) for financial support.

References

Al-Hussaini, T. K., Abdelaleem, A. A., Elnashar, I., Shabaan, O. M., Mostafa, R., El-Baz, M. H., El-Deek, S. E. M., & Farghaly, T. A. (2018). The effect of follicullar fluid pesticides and polychlorinated biphenyls concentrations on intracytoplasmic sperm injection (ICSI) embryological and clinical outcome. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, *220*, 39-43. https://doi.org/10.1016/j.ejogrb.2017.11.003

Berge, A. C., & Vertenten, G. (2017) Whitepaper: The importance of preventive health and vaccination programmes in ruminant production. Available from https://www.timetovaccinate.com/. Accessed in: July 5th. 2022

Blondin, P., Vigneau T, C., Nivet, A. L., & Sirard, M. A. (2012). Improving oocyte quality in cows and heifers - What have we learned so far? *Animal Reproduction*, *9*(3), 281-289.

Cabry, R., Merviel, P., Madkour, A., Lefranc, E., Scheffler, F., Desailloud, R., Bach, V., & Benkhalifa, M. (2020). The impact of endocrine disruptor chemicals on oocyte/embryo and

clinical outcomes in IVF. *Endocrine Connections*, *9*(6), 134-142. https://doi.org/10.1530/EC-20-0135

Chefer, D. M., & Souza, D. M. (2016). Bovinocultura de corte: principais manejos durante a fase de cria. *Revista Iniciare*, *1*(1), 1-12.

Clarke, H. G., Hope, S. A., Byers, S., & Rodgers, R. J. (2006). Formation of ovarian follicular fluid may be due to the osmotic potential of large glycosaminoglycans and proteoglycans. *Reproduction, 132*, 119-131. https://doi.org/10.1530/rep.1.00960

De Loos, F., Van Vliet, C., Van Maurik, P., & Kruip, A. M. (1989). Morphology of immature bovine oocytes. *Gamete Research*, *24*, 197-204. https://doi.org/10.1002/mrd.1120240207

European Medicines Agency – EMA. Guideline for Good Clinical Practice Londres, 2017.

Ferreira, T. P., Lima, I. P., Magalhães, V. S., Avelar, B. R., Oliveira, G. F., Scott, F. B., & Cid, Y. P. (2019). Bioanalytical Method to Measure Fluazuron in Bovine Plasma and its Application in Pharmacokinetic Studies. *Revista Virtual de Química*, *11*, 1067-1079.

Gordon, I. R. (2003). Laboratory Production of Cattle Embryos (Biotechnology in Agriculture). 2nd rev ed. Wallingford, UK: CABI Publishing.

Gusmão, A. O. M., Silva, A. R., & Medeiros, M. O. (2017). A biotecnologia e os avanços da sociedade. *Biodiversidade*, *16*(1), 1-20.

Hermida, C., Pelaez, V., & Silva, L. R. (2015). Limites de Residuos de plaguicidas y barreiras técnicas de comércio. *Revista agroalimentaria*, *21*, 151-170.

InternationaL Programme on Chemical Safety - IPCS. Toxicological evaluation of certain veterinary drug residues in food. Fluazuron: WHO Food Additives series 39. Geneva:WHO (World Health Organization), 1997.

Mapholi, N. O., Marufu, M. C., Maiwashe, A., Banga, C. B., Muchenje, V., Mac Neil, M. D., Chimonyo, M., & Dzama, K. (2014). Towards a genomics approach to tick (Acari: Ixodidae) control in cattle: a review. *Ticks tick-borne disease*, *5*(5), 475-483. https://doi.org/10.1016/j.ttbdis.2014.04.006

Sartori, R., Gimenes, L. U., Monteiro, P. L. J., Melo, L.F., Baruselli, P. S., & Bastosd, M. R. (2016). Metabolic and endocrine differences between *Bos taurus* and *Bos indicus* females that impact the interaction of nutrition with reproduction. *Theriogenology*, *86*(1), 32-40. https://doi.org/10.1016/j.theriogenology.2016.04.016

SAS - Statistical Analyses System. Version 8.01. Cary, NC: 2000. (Online manual).

Shalgi, R., Kraicer, P., Rimon, A., Pinto, M., & Soferman, N. (1973). Proteins of human follicular fluid: the blood-follicle barrier. *Fertility and Sterility*, *24*, 429-434. https://doi.org/10.1016/S0015-0282(16)39730-8

Sohel, M. M. H., & Cinar, M. U. (2014). Advancement in Molecular Genetics to understand the molecular reproduction of Livestock follicular development. *Research in Agriculture, Livestock and Fisheries*, *1*(1), 47-60. https://doi.org/10.3329/ralf.v1i1.22355

Swindale, M. S., Sibson, G., Bull, M. S., & Hess, E. A. (1993). Control of bovine ticks in humid tropical Queensland with ACATAK Pour-on. Unpublished report No. 93/3/1384. Ciba-Geigy Limited, Kemps Creek, Australia. Submitted to JECFA by Ciba-Geigy Limited, Basle, Switzerland.

Taninaka, T., Bernardino, T., Meneghini, R., & Santos, G. (2015). Análise da viabilidade econômica de um rebanho de gado de corte da raça Wagyu em ciclo completo. *Revista iPecege*, *1*(2), 44-58.

Thomas, P. L., Swindale, M. S., & Hess, E. A. (1992). Persistência do efeito acaricida do fluazuron, aplicado em formulação pour-on a 1,5 e 2,5 mg/kg, contra *Boophilus microplus*. Ciba-Geigy Limited, Kemps Creek, Australia.

Xin-Lun, L., Yu-Mei, Z., Cheng-Yun, H., Wei-bo, C., Xue, C., & Ming-Rong, Z. (2013). Pharmacokinetics of fluazuron in cattle. *Chinese Journal of Preventive Veterinary Medicine*, *10*, 1595.

Zamai, M. F., Cavalieri, F. L.B., Andreazzi, M. A., Morotti, F., Seneda, M. M., Santos, J. M. G., Moreski, D. A. B., & Colombo, A. H. B. (2021). Synchronization with estradiol benzoate in the presence of the *corpus luteum* in Wagyu cows increases the number of medium follicles but does not interfere with in vitro production of embryos. *Semina: Ciências Agrárias*, *42*, 1147-1158. https://doi.org/10.5433/1679-0359.2021v42n3p1147

Zhang, Y., Lu, X., Hu, C., Cui, W., & Liu, X. (2013). Development of a high-performance liquid chromatography method for quantification of fluazuron in cattle tissues. *Journal of Liquid Chromatography & Related Technologies*, *36*, 2559. https://doi.org/10.1080/10826076.2012.723090

Zoetis (2022). Tackzuron pour-on - Technical information - Technical data.