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The works must be unpublished and refer to topics of Experimental design, commerce, options, rural-flora and fauna, agronomy, natural and other topics related to Biology and Chemistry.

Presentation of the Content

As a first chapter we present, *The craft sector: an approach to their identity and trade*, by SERRANO-REYES, Germán Francisco & GORDILLO-BENAVENTE, Liliana de Jesús, with adscription in Universidad Politécnica de Tulancingo, as a second article we present, *Direct infiltration effects of dimethyl sulfoxide into ovarian bursas on spontaneous ovulation and the estrous cycle of the rat: antagonism on the type 2 dopaminergic receptor*, by MORÁN-PERALES, José Luis, OLVERA-HERRERA, Jasiel Evani, HANDAL-SILVA, Anabella and GARCÍA-SUÁSTEGUI, Wendy Argelia, with adscription in Benemérita Universidad Autónoma de Puebla, as the third chapter we present, *Phytopathogenic fungi in seeds of the genus Pinus, stored in a gene bank*, by AVENDAÑO-LOPEZ, Adriana Natividad, GONZALEZ-FLORES, Mario Israel, ROMÁN-MIRANDA, María Leonor and SÁNCHEZ-MARTÍNEZ, José, with affiliation at Universidad de Guadalajara, as fourth article we present, *Effect of inoculation with mycorrhizal fungi on the production of native corn (Zea mays L.) in Valle de Santiago, Gto*, by VARGAS-ESPINOZA, Everardo, GAYTÁN-RUELAS, Marina, CALDERÓN-RUIZ, Alberto and MARTÍNEZ-CAMACHO, Adriana Paola, with adscription in the Universidad Tecnológica del Suroeste de Guanajuato.

Content

Article **ECORFAN Journal-Ecuador**
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The craft sector: an approach to their identity and trade

El sector artesanal: un acercamiento a su identidad y al comercio

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Abstract

The objective of this article is to analyze from a critical perspective the cultural identity and the commercialization of the artisanal sector in Mexico making an international comparison. Based on the fact that "handicrafts" are the identity of a town, community, person or country, on many occasions this "Identity" is lost when people called "intermediaries" take advantage of artisans and buy their merchandise at low cost, to sell them obtaining important profits at the expense of the work of the artisans; the "intermediary" is only interested in trade, sales, profits; that is to say, it only cares about the merchandise, without giving any value to the essence of the crafts than the cultural identity of who created it. The methodology of this research was qualitative with a documentary technique where articles were reviewed through ABI/INFORM Global Google Scholar, obtaining as results 12 articles that the words or keywords referred to. The main findings were that throughout Latin America it is observed that the artisanal sector is penalized in terms of costs and that it occurs more in the form of intermediaries instead of direct sales and this results in the artisanal sector not being valued as a town identity.

Craft sector, Trade, Identity

Resumen

El objetivo del presente artículo es analizar desde una perspectiva crítica la identidad cultural y la comercialización del sector artesanal en México haciendo una comparación internacional. Partiendo de que las “artesanías” son la identidad de un pueblo, comunidad, persona o país, en muchas ocasiones se pierde esta “Identidad” cuando personas llamadas “intermediarios”, se aprovechan de los artesanos y les compran su mercancía a bajo costo, para venderlas después obteniendo ganancias importantes a costa del trabajo de los artesanos; el “intermediario”, sólo le interesa el comercio, la venta, las ganancias; es decir, solo le importa la mercancía, sin darle valor alguno a la esencia de las artesanías que la identidad cultural de quién la creó. La metodología de esta investigación fue cualitativa con una técnica documental donde se revisaron artículos a través de ABI/INFORM Global google Académico obteniendo como resultados 12 artículos que las palabras o keywords hacían referencia a este. Los principales hallazgos fueron que en toda Latinoamérica se observa que el sector artesanal está castigado en cuanto a costos y que se da más de forma de intermediarios en lugar de la venta directa y esto trae como consecuencia que no se le de valor al sector artesanal como identidad del pueblo.

Sector artesanal, comercio, identidad

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Introduction

Article 2 of the Political Constitution of the United Mexican States currently contemplates and recognises the existence of "indigenous" peoples as part of the country's cultural plurality. In other words, it raises to a higher hierarchy of norms the conformation of a Mexico in which various ways of being, thinking and acting are integrated; a reason that gives modern Mexico a social reality; not only as it had been made to feel, that the thinking and way of life of Mexicans was only one. Therefore, the plurality among the social groups that make up the Mexican community is varied; and, in recognition of this, the Constituent Assembly stipulates and decides so (Carbonell, 2004).

In this regard, our Supreme Law determines that: "Awareness of their indigenous identity shall be a fundamental criterion for determining to whom the provisions on indigenous peoples apply" (Unión, 2006); this is a significant advance in the vision of equality, given that the "identity" of indigenous groups is recognised by it, fulfilling the historical commitment to put them on an "equal" footing when the administrative authority has to resolve controversial issues or questions of application of the Law. In other words, when the administrative authority has to apply norms or regulatory provisions, it will do so with full knowledge of the way of life, culture and language of the people belonging to the so-called "indigenous peoples".

Furthermore, our Magna Carta recognises that the reality of Mexican society is one and that which is embodied in the norm is another, which is why "contrariu sensu" it stipulates in its content that this is not the case in daily life and that it needs to be integrated in writing so that the authorities, above all, avoid discrimination against this sector of the population at all costs.

In the same constitutional precept in its fourth paragraph, the constitutions of the federal states must also recognise indigenous peoples and communities and their autonomy, which states: the recognition of indigenous peoples and communities shall be made in the constitutions and laws of the federal states, which shall take into account, in addition to the general principles established in the previous paragraphs of this article, ethno-linguistic and physical settlement criteria (Unión, 2006). (Unión, 2006).

It is clear, then, that the constituent has managed to elevate equality in Mexican society to constitutional rank, through the express recognition that the basis of society itself are the "indigenous peoples". The question then arises: what is constitutional recognition?

Constitutional recognition of indigenous groups is equivalent to institutional recognition, i.e. the acceptance of the existence and the rights and obligations of a sector of the population by the state, not only in a political discourse, but to raise it to constitutional rank is to place public institutions at the service of this sector of society, to safeguard those rights and enforce compliance with state norms; and if the Constitution itself obliges public institutions to such recognition, it also obliges them to promote their way of life.

Since 2000, there has been significant progress in the world with regard to the recognition of indigenous peoples, especially in the United Nations, where one of the important drivers of the social movement was the roundtable entitled "Economic Empowerment of Indigenous Peoples of Latin America and the Caribbean" in 2017 and especially the "United Nations Declaration on Indigenous Peoples", Convention 169, published by the International Labour Organization (ILO) in 2014. (UNITED NATIONS, 2014).

Research problem

In Mexico there is a great problem of not giving the credit that the artisan sector deserves. Undoubtedly, "middlemanism" is a necessary evil, but at the same time it is a challenge for the handicraft industry on a small scale; and at the same time, the artisan lacks market vision and there are some who, in addition to creating the handicrafts, market them directly; but unfortunately to do so, they have no choice but to sell their products on the streets, pavements, in a forum, or at a town or community fair; and the "crafts" as a representation and manifestation of the "cultural identity" of who created them, are unique pieces, but for sale to "intermediaries", they mass produce their products, and only that cultural expression remains for the artisans; and the final buyer, sees in these products the style, the ornamental aesthetics or practical use or satisfaction of daily needs, without appreciating the cultural traits of their creator; this phenomenon is not typical of Mexico (Bustos, 2017) and (Cerdas, 2010).

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In the same idea, it is convenient to know, grosso modo, the diverse ways of life of the Mexican population, in times of pandemic and economic globalisation; globalisation that focuses on the economy and finance, but in the background is a change of thinking towards the uniform determination of large commercial corporations that have invaded the world, but with it the national production has fallen substantially in all sectors; but in reality it is the "Intermediaries", to a large extent, who have been economically impacted; but as most of the craft industry relies on them, the artisans have been impacted in the sale of their products, as a final result. But it is necessary to have some elements of the products themselves for their commercialisation; the question arises: What do "handicrafts" need in order to be sold in large departmental chains? Therefore, this research focuses on analysing from a critical perspective the cultural identity and the commercialisation of the handicraft sector in Mexico, making an international comparison.

Literature review

The original (indigenous) peoples of Mexico

According to the "Sistema de Información Cultural" of the Government of the Republic, there are currently 71 indigenous peoples, distributed as follows:

State	Municipality/Locality	Name
Baja California	Ensenada	Ku´ahles Cochimíes Pa ipaís Kiliwas
	Mexicali	Cucapás
	Tecate	Kumiais
	Texistepec	Texistepequeños
Campeche	Campeche	Ixiles Q'eqchis'
	Champotón	K'iches Awakatekos
Coahuila	Coahuila	Kikapúes
Chiapas	Amatenango de la Frontera	Jakaltekos Kaqchikeles Mames
	Las Margaritas	k'anjob'ales- Q'anjob'ales Tojolabales
	Mazapa de Madero	Tekos
	Motozintla	Mochós
	Ocosingo	Tzeltales Lacandones
	Rayón	Zoques
	La Trinitaria	Chujes Akatecos
Chihuahua	Guachochi	Tarahumaras
	Guadalupe y Calvo	Tepehuanos del norte
	Madera	Pimas
	Uruachi	Guarijó
Ciudad de México	Ciudad de México	Nahuas
Durango	valle de Canatlán	Tepehuanos del sur
Guanajuato	Guanajuato	Chichimecas
Guerrero	Ometepec	Amuzgos
	San Luis Acatlán	Tlapanecos
Hidalgo	Valle del Mezquital	Otomíes
Estado de México	Ixtlahuaca	Mazahuas
	Ocuilan	Tlahuicas
	Temascaltepec	Matlatzincas
Michoacán	islas del Lago de Pátzcuaro	P'urhépechas
Nayarit	Del Nayar	Coras
	La Yesca	Huicholes
Oaxaca	Huautla de Jiménez	Mazatecos
	Juchitán de Zaragoza	Zapotecos
	Oaxaca de Juárez	Mixtecos
	San Martín Itunyoso	Triquis
	San Mateo del Mar	Huaves
	Santa María Ixcatlán	Ixcatecos
	Santa María Nativitas	Chocholtecos
	Santa María Zacatepec	Tacuates
	Santiago Yaitepec	Chatinos
	Unión Hidalgo	Mixes
	San Juan Bautista Valle Nacional	Chinantecos
Puebla	Tehuacán	Popolocas
San Luis Potosí	Ebano	Huastecos
	Santa Catarina	Pames
Sonora	Alamos	Guarijíos
	Cajeme	Yaquis
	Hermosillo	Seris
	Huatabampo	Mayos
	General Plutarco Elías Calles	Pápagos
Tabasco	Jalpa de Méndez	Ayapanecos
Veracruz	Filomeno Mata	Totonacos
	Ixhuatlán de Madero	Tepehuas
	Oluta	Olutecos
	Sayula de Alemán	Popolucas Sayultecos
	Texistepec	Texistepequeños
Yucatán	Macuspana	Chontales
	All the territory	Mayas

Table 1
Source: Own elaboration

The population of indigenous peoples is 23.2 million people nationwide, ((INEGI), 2022); and there are 68 native languages (REPÚBLICA, 2021). The original peoples, also called "indigenous peoples", have "uses and customs" that are specific to their way of life, their environment and their interpretation of the world, or "Cosmovision".

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Culture

The concept of "culture" is defined from different points of view; the anthropological one, according to Podestá (2006), determines that Edward B. Taylor defines it as "...". Taylor defines it as "... that totality that includes knowledge, beliefs, art, morals, law, customs and any other aptitudes and habits that man acquires as a member of society"; it can be appreciated from the concept that the way of life of people as individuals or as a group is the central axis that makes "culture"; Each person who integrates a social group, is made to its rules, but also to its way of life where "art" is emphasised, but without pretending to separate it from the community as a whole; and it is where it can be deduced that "art" is undoubtedly the expression of all its knowledge, beliefs, morals and customs as a form of individual and social life.

From sociological thought, "culture", according to Murguía (2002), citing Emile Durkheim, is defined as "a set of social phenomena".

According to social psychology, "culture" is: a system of interrelationships between individual ontogenetic processes, social and historical processes of collective behaviour in a given period of time, and anthropological and historical processes that make cultural products possible, including artistic, everyday, scientific, technological and folkloric manifestations (Vera, 2009).

From the above definitions, it can be seen that the foundation that gives the character of "culture" is the thinking of a social conglomerate; its interpretation of life and the subjective understanding of its natural environment and its worldview of the encounter with its deities. It is also to rescue from the previous statements that they are assumed behaviours, which give them that sense for life and for keeping active naturally.

According to the declaration of the United Nations Educational, Scientific and Cultural Organisation (UNESCO), which defines "culture" as: the set of distinctive spiritual, material and emotional features that characterise a society or social group. It encompasses, in addition to arts and literature, ways of life, fundamental human rights, value systems, beliefs and traditions (Molano, 2007).

Therefore, "culture" is a harmonious whole, it is that which distinguishes a people from others, with its characteristic stamp of unique manifestations; assumed knowledge that is transmitted from generation to generation in that social group. Peoples are distinguished by their way of life which gives them "identity", and makes them unique in the social sphere where they belong and interact; such as "indigenous peoples".

"Culture" and Law in Mexico.

The philosophical vision that makes law possible is society. The behaviour of individuals as they interact with other members of the group. Therefore, the purpose of Law is the "social order"; that is, to maintain the individuals of a society in a structure through rules of behaviour and institutions of the State, for its study and solution in case of "conflicts" when necessary (Gonnet, 2018).

As it is not the object of study of the present work, the relationship between culture and Law, we will not delve into the subject; but it is worth noting that according to Ihering's definition of Law, which gives meaning to it, it is the so-called "spirit of Law" which in his work determines that it is the same as being the foundation of Law and interpreted as the "ideas, conceptions, tendencies of a people and of an era", in short it can be understood as Law being the product of the "thinking of a people". (Rodriguez, 1987).

Regarding the term "culture" it has been seen from three perspectives; the first as a derivation of the word "cultivation" which would be the cultivation of the land, and in metaphorical form it would be "the cultivation of the human being", by itself and in society, protected by the nature of the word that its base is "the cult", understood as "knowledge"; the second as "set of knowledge to develop a critical judgment" and the third as "set of ways of life and customs, knowledge and degree of artistic, scientific, industrial development, in an era and social group" (Hurtado, 2011, Hurtado, 2011). (Hurtado, 2011).

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Cultural identity

Identity in the words of Molano (2007) as: Identity is only possible and can only be manifested on the basis of cultural heritage, which exists beforehand and its existence is independent of its recognition or valuation. It is society that, as an active agent, configures its cultural heritage by establishing and identifying those elements that it wishes to value and assume as its own, and those that naturally become the referent of identity (Molano, 2007).

According to what has been described, "cultural identity" is made up of two linguistic meanings, "heritage" and "culture".

It can be seen that "heritage" according to the concept of the Royal Academy of the Spanish Language is: "set of goods and rights acquired by any title" (Spanish, 2021). (Spanish, 2021).

Thus, "cultural identity" can be integrated, on the one hand, of physical or natural heritage and on the other hand, intangible heritage; according to the analysis of (Cepeda, 2017) "natural heritage", (...), is constituted by physical, biological, geological and physiographic formations that make up the habitat of endangered species and that present an exceptional universal value and natural places with an exceptional universal value from the point of view of science, conservation or natural beauty. Intangible cultural heritage" means the practices, representations, expressions, knowledge and skills, together with the instruments, objects, artefacts and cultural spaces associated therewith.

From an anthropological point of view, there are two visions of "cultural identity", the "essentialist current" and the "constructivist current"; the former assumes that cultural traits are transmitted through generations and that, at the same time, they shape "cultural identity"; the latter states that identity is something that is constructed, ignoring the first current, which can be understood as something that is inherited (Martínez, 2008).

These points of view have something in common; the determination that "cultural identity" exists, "cultural identity" is drawn from what is described, which is undoubtedly the "expression of the sensitive spirit of the beliefs of some people"; "that which is there, but which is not seen"; that immateriality subsumed and expressed in different forms, with words, with gestures, with bodily attitudes, as gestures, among others, and sometimes in materials, which give unique value to such expressions, because it represents both the material and immaterial cultural heritage of each person, group or society.

The "cultural identity" can only be imagined, but the person or human group that lives it, is the one that has the exclusivity to do so, because I repeat, they live it; it is among them; although, their beliefs are for some even fanciful, as a discriminatory trait, it reflects their ignorance in not understanding that "immaterial cultural heritage" of the people.

In this sense, we all have a "cultural identity", even the scientific and technological work; it is remembered with nostalgia, that phrase of our ancestors who said "the sun rises for everyone"; which made it known that, in a society, each person or group has their own space. For example, science and technology are currently gaining space among the people, and it cannot be denied that even in spaces where it was not known, today the use of technology, derived from modern science, has intrusion; but the cultural identity of indigenous peoples also has its own space, and also, although not with the same expansion as science and technology, it is gaining space not only in Mexico, but in Latin America; Each "cultural identity" has its own independent space, without falling into dramatism, in expressions such as "indigenous peoples are going to disappear because of science and technology, which is relegating them", is not entirely correct, because as part of the reflection, "cultural identity" is not lost, it is the spaces that move, and specifically, the cultural identity of indigenous peoples will never be lost. (Lechner, 2002), (Jullien, 2017).

In reality, each society processes, combines and rearticulates the elements that circulate at the global level in a specific way. This appropriation and "nationalisation" of global processes affects not only social ties and habits, but also familiar mental schemas.

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In this sense, the "cultural identity", especially of the original peoples, will not lose its appreciation by many people, not only where they come from and are owners of their cultural heritage, but the recognition of their way of life (beliefs, traditions, values, morals, way of thinking) is gaining more and more space among the Mexican population; as mentioned, the original peoples of Mexico, such as the Afro-Mexican groups, with all their "culture", are already recognised.

Again, it is a significant step forward to give indigenous and Afro-Mexican peoples a deserved place of social equality in a modern society with cutting-edge scientific and technological advances, as part of people's lives in general; nowadays, it is common to see people from indigenous groups using mobile phones, going to universities, using electronic technologies, etc., but this does not mean that "cultural identity" is being displaced.

According to Mansilla "is that the concept of identity "refers to a notion of ourselves, in function or in comparison with others who are not like us" (Mansilla, 2006), that is to say; it is what identifies us, gives us identity, makes us different from others, that which makes us original, or with our own identity, which only materialises when the individual is in society; therefore, it could be determined that "Identity" is the manifestation of what one is (Ranaboldo, 2007). And, when this "Identity" refers to the way of life and the "cosmovision" of the environment, natural or divine, the "Cultural Identity" is born; and, consequently, the "Cultural Identity".

The doctrine affirms that "Cultural Identity" is no longer a way of arriving at the pure concept of peoples, due to the migratory society from one people to another, from rural communities to big cities, from one country to another; migration that is produced by diverse socio-economic and political factors, which will not be dealt with in the present work (Vallespir, 1999).

As a consequence of the migration of people, the so-called "cultural diversity" has been produced, a concept that UNESCO has pronounced as "heritage of humanity" (Álvarez, 2013). There is no doubt that for anthropologists it is an extremely complex subject; however, Schmidhuber's proposal appears which determines the demonstration of a culture, starting from a "symbolic object", giving an approach to the definition of culture of Lévi - Strauss; that understands as the "culture, is formed from the symbolic meanings that the members of a community give to the objects", this meaning has great relevance for the present work, since the objects that bring subsumed the representations of diverse meanings, is the expression of a culture, a way of life, a way of seeing the world, to which we denominated "Craftsmanship". (Amaya, 2002)

Crafts

The productive activity is "creativity" (Gastón, 2011). Creativity" is one of the most controversial concepts, ranging from "genius" to the potential to create something, passing through "inventiveness", "production" and "discovery", according to various authors cited by: (Chacón, 2005); the points of view are exposed in parts; the first is from the approach of the person as attributes of herself; and the second from the result or expression of the person; without any doubt, "creativity" is inherent to the person, which is related to the ability to reflect and solve a problem, to the ability to produce an object or something recognised by others.

Therefore, "creativity" is a necessary element coming from the cognitive process, which allows, on the one hand, to "create an idea" and then, on the other hand, to express this creative capacity with verbal or material manifestations or expressions (Diez, 2009). It is necessary for the present work, that the "creativity" referred to is human creativity, without distinction of lifestyle, economy, schooling, and other factors that socially could make people different from their perception; that is to say, "creativity" does not have much or nothing to do with social issues, but it is the capacity of people to create; that according to ways of life, economic, cultural, spiritual systems, are accepted and even questioned by some and accepted by others; but they are not without reason to deny that people do not have this, which is characteristic of them.

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Each person or social group determines the ideas, customs, traditions, religion, etc., that they will accept as a personal or social norm that allows them to express this "creativity", as in the case of "handicrafts", and therefore handicraft work is a social activity, influenced by economic relations, cultural relations and social, political and even religious reorganisation of the social group itself (Vega, 2019). It is the manifestation of their way of life and their beliefs (Vega, 2019).

This intrinsic part of the human being is today recognised as "intangible cultural heritage" as we have already discussed; and crafts, far from being a decorative or ornamental object, as we have tried to see, is the expression of a way of life of some people or groups of people, which are not related to the economy, science and technology, as has also been mentioned; This is because they only have their very special way of life, a style that gives them their "cultural identity", which makes them different from others in their way of life; as valuable and as important as any other.

Therefore, the term "crafts" has had several connotations over time among the states of Latin America; for example, in Brazil since 1988, in its Constitution, the "cultural heritage" has been recognised, both tangible and intangible, where "crafts" are found (Pérez, 2012); In Peru, in addition to being recognised by its Constitution, the Ministry of Foreign Trade and Tourism is also delegated the promotion of handicrafts in that country, and its legal regulations classify them into "Traditional Handicrafts" and "Innovative Handicrafts"; the former "are goods that have a utilitarian, ritual or aesthetic use and represent the customs and traditions of a given region. They constitute, therefore, a material expression of the culture of communities or ethnic groups". And the second as: "They are goods that have a functionality, generally of a decorative or utilitarian nature, which is highly influenced by market trends"; also in Peru there is a "National Register of Artisans" that gives recognition to the person as a craftsman or craftswoman; (Alcántara, 2018) in Colombia there is the "Cámara de Comercio de Pasto y el Laboratorio Empresarial de Artesanías", which is responsible for the international marketing of handicrafts, and the concept of handicrafts is based on the UNESCO definition,

"The special nature of handicraft products is based on their distinctive characteristics, which may be utilitarian, aesthetic, artistic, creative, linked to culture, decorative, functional, traditional, symbolic and religiously and socially significant" (Ortiz, 2015).

In addition to the above, in Venezuela, the "Departamento Nacional de Planificación" (National Planning Department) has classified handicrafts since 2006 as follows: a) "indigenous crafts" which are the expression of these communities that are transmitted from generation to generation; b) "traditional crafts" those made by mestizo and black communities, with technique influenced by European migrations; c) "contemporary crafts" those that include technical and aesthetic elements of the social, economic and cultural context; d) "popular art" individualised productions in which creativity is materialised; e) "ethno-crafts" which are those inherited in the same way as "indigenous crafts"; f) "semi-industrialised crafts" their production is urban as a result of the practice of a trade or specialised schools. (Bustos, 2009).

Along the same lines, in Ecuador there is the "Centro interamericano de artesanías y arte popular CIDAP located in Cuenca - Ecuador", which is responsible for raising awareness of the world of crafts, and perceives crafts as the product that carries aspects inherent to a cultural identity (Mendieta, 2020).

Bolivia, for its part, determines that "crafts" is the object: "fundamentally manual production carried out with or without the aid of machinery, an activity that can be carried out individually or through associations and/or cooperatives as independent economic units"; (Alcántara, 2018).

In Argentina, according to its Secretariat of Culture, it gives them the following denomination: "those products "...produced with artistic intention and/or destined to fulfil a utilitarian function, in a predominantly manual form; whose design is representative of the cultural diversity of the Argentine Republic" (Pastor, 2006).

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As for Mexico, nowadays, its control, promotion and registration is established to the Secretariat of Culture, in accordance with the attributions granted by the Organic Law of the Federal Public Administration; and the concept of "folk art" has been created to refer to "handicrafts" and recognises that they are: "the natural wealth used for the elaboration of handicrafts, that is to think of a variety of woods, fibres, skins, resins, etc., an enormous diversity of materials used in the elaboration of folk art", an enormous diversity of materials used in the manufacture of folk art" National Commission for the Knowledge and Use of Biodiversity (CONABIO, 2022).

There is no doubt that the common denominator of the concept of "handicrafts" is "cultural identity", which identifies each people, group or community or country, in its way of life, its way of thinking, its beliefs and traditions, which make it unique; That which is there and cannot be seen, but which is proper to the person, the group, the people, the community; and which is an aspect that must be studied in depth to understand its culture, its way of life; and let us be sure that once it has been analysed and lived, it will be given the value it has, which does not depend on others, which only comes from its own nature.

It is convenient to say that in Mexico, and mainly in the State of Oaxaca, they added the Coronavirus (Covid19) to their artistic creations, such as the masks they use in their rituals (Hernández, 2020).

Intermediaries are the people who go to homes, workshops or other places to buy figures at lower prices and resell them, or the artisans go to offer them to traders in markets (Rivera Cruz, 2008).

Starting from the fact that the "crafts" are the identity of a town, community, person or country, in many occasions this "Identity" is lost when people called "intermediaries", take advantage of the craftsmen and buy their merchandise at low cost, to sell them later obtaining important profits at the expense of the work of the craftsmen; the "intermediary", is only interested in the commerce, the sale, the profits.

That is to say, only the merchandise matters to him, without giving any value to the essence of the crafts that the cultural identity of who created it; the intermediary also takes advantage of the little or null chain of distribution of the craftsmen, in many occasions, by the rural geographical zone where they live, and to the craftsman to survive, he sells to the highest bidder his work and creations, designs and culture, at insignificant prices. (Jeannine Uwimabera, 2017).

But it is necessary to have some elements of the products themselves for their commercialisation; the question arises: What do "handicrafts" need to be sold in large departmental chains? The answer to this question can be given in a more extensive work, but roughly speaking, it is to comply with the minimum standards required by the chains; then, according to the above, it is only possible to comply with such requirements, to the "intermediaries", because it is reiterated, the artisans, are not interested in marketing their products on a large scale, because their primary idea is to capture in materials their "Cultural Identity" beyond whether they sell or not (Calvo, 2011, p. 19). The sale of products in general is carried out in three different ways, traditional, modern and electronic.

In Mexico from the modern channel, there are three large market chains that cover the whole country; called "self-service shop chains", with the format of "Bodega, supermarket, hypermarket and megastore (BSHM), which are Walmart, Soriana and to a lesser extent Chedraui". Walmart has a presence in 87% of the country's geographic areas, and in half of these, there is no competition for shops, through large investments, distribution chains, distribution centres (CEDIS), and the use of information and communication technology. Federal Economic Competition Commission ((COFECE), 2020).

Sales strategies, as explained above, apparently do not interest the artisan, because their purpose is not trade, but to express their ideas and ways of life of their community, village or person, so these strategies are left to the "middleman", who is the one who carries them out in some markets, once he has exploited the designs, craft creations that he did not elaborate.

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In the opinion of various authors that have been reviewed, that science and technology, and now we add the importation of various articles that, by the way, there is a confusion between "handicrafts" and "crafts" as already mentioned; and it is thought that the imports of "handicrafts" harm the crafts, sending them more and more towards their extinction; but this is a false belief; crafts do not depend on the market, they depend on the "intermediary", not on the craftsman; he will never stop creating and designing them, as long as his intentions do not stop expressing his own "cultural identity", which will never disappear, as long as there are people with this way of thinking and living.

The export of handicrafts

International trade treaties in the five continents have been very useful for traders of different economic levels; without a doubt, it has benefited trade, defining it as: "legal acts that contain commercial speculation" according to the Mexican Commercial Law, (Calvo, 2011); that is, they are the so-called "traders", which are the physical or moral persons that carry out the so-called "acts of trade"; but, it is worth asking, is the artisan sector included as a trader? The answer is no; as we have been exploring, the so-called "traders" are the persons who have the purpose of being in commerce, that is to say, they are the persons who manufacture, produce, sell or acquire goods or services, but already before they do so, they already have the objective of commercial speculation.

In this sense, the handicraft sector, it is emphasised, does not aim to speculate in trade, but to express their ideas, beliefs and ways of life in some materials, such as stone, leather, fabric, wood, glass, among others. Therefore, international trade treaties exclude the handicraft sector, as it is considered a non-commercial sector (Hernández Ramírez, 2011).

It should be noted that in the craft trade sector, it is the "intermediaries" who can benefit from these international trade treaties, but it is not possible for the artisan, because international trade treaties also aim to make possible the "traffic of goods" in a globalised world, with standards defined by the countries involved in this traffic. International standards such as the international terms of trade (INCOTERMS), for the standardisation and logistics of the transport of goods; homologation of technical aspects, of materials, classifying them in different ways, by their use, by their workmanship, their destination, procedures and documents, etc., which makes the world of trade as uniform as possible, throughout the world; respecting the technical and environmental decisions that restrict the handling of both the import and export of these goods.

Therefore, the export of "handicrafts" has a different aspect to the so-called "merchandise", but it would undoubtedly help to combat the economic poverty in which the authors and creators of handicraft designs and products find themselves; it is necessary to include "handicrafts" as an exportable product, but by their creators who are the artisans and not only by the "intermediaries".

Countries exporting "handicrafts"

In this regard, it should be noted that some countries in Latin America and other continents have long had the regulatory conditions in place in their governments to support the export of handicrafts, but it is not clear whether or not the person who makes them is an artisan, with research focusing only on the exportable product without specifying whether the exporter is one or the other.

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Such is the case of African countries, with their embroidery, furniture, textiles and vegetable dyes, exported to Germany, France and Switzerland; the case of Asia, such as Korea, with its cloth and cotton embroidery; of China, with its ancestral hand-weaving techniques in cotton, silk and hemp; in Malaysia with its vegetable dyes; in the Arab States, with its designs in silk or velvet dresses; in Europe, especially Spain, with its wooden furniture, jewellery, costume jewellery, textiles, ceramics and leather goods; in Portugal, with its embroidery, silk, silk and hemp; and in Spain, with its wooden furniture, jewellery, jewellery, textiles, ceramics and leather goods; in Portugal, with its embroidery, lace, basketry, cabinetwork, cork and gold and silver filigree; in Latin America, especially in Argentina, with its traditional weavings of hand-dyed and hand-spun sheep's wool tapestries; in Cuba, with its wood and leather sculptures; in Colombia, with its weavings and hammocks, palm mats, chinchorros, wicker products, wood, jewellery and leather; in Peru, with its alpaca products; in El Salvador, with its products made of clay, natural fibres, wood, seeds and fabrics; among other countries. (Hernández Ramírez, 2011).

Because of the above, it is very necessary for Mexico to open up to the export of its handicraft products, but in addition to what the "intermediaries" do, because in the end it is the sale of "handicrafts", but now, the export must be carried out by the owners of the "cultural identity", which are the artisans.

Conclusion

It is indispensable to make the distinction that the "crafts" are those that have incorporated the "cultural identity" of those hands that created them; "cultural identity" that is characterised by making known and manifesting their "culture" but also by identifying the customs, the environment in which they live, their beliefs, their rites, traditions, in a clear "cosmovision" of the environment in which they live on a daily basis.

Identifying the culture of people, towns, communities, in products, called "crafts", is not only an ornament, souvenirs, ornament; it is the way of life of people who express what they feel, live, and proudly express, because they also feel proud of their ancestors, cultivators of the same customs transmitted from generation to generation, no matter if others are interested in their products, they have already achieved their unique purpose, which is to express their ideas in materials.

Therefore, artisans do not depend on technology and its advances, on science and its demonstrations, on the social, political and economic acceptance or rejection of some; they are independent beings, free of thought and life; as long as there is an ancestral way of life, there will be culture and therefore, "crafts"; they do not depend on competitiveness, innovation, and industrial production, among other economic forms; artisans have their own way and it is reiterated, independently.

It is indisputable that the world has managed to nationalise the way in which foreign trade is conducted, this is a great commercial advance; and its achievements have allowed countries to expand their trade abroad and to obtain goods that they do not have or are scarce in their territories; It is undoubtedly an effective commercial strategy, despite the deterioration of the environment, where the trader has been able to survive; and it is now necessary to open the borders to exchange handicraft products such as those of Mexico, but from the perspective of carrying out international trade operations by artisans, the sole creators and owners of their "Cultural Identity"

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Direct infiltration effects of dimethyl sulfoxide into ovarian bursas on spontaneous ovulation and the estrous cycle of the rat: antagonism on the type 2 dopaminergic receptor

Efectos de la infiltración directa de dimetil sulfóxido dentro de las bursas ováricas sobre la ovulación espontánea y el ciclo estral de la rata: antagonismo del receptor dopaminérgico tipo 2

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Abstract

We studied effects of microinjection (MI) into ovarian burses (OB) of infiltering agent dimethyl sulfoxide (DMSO) on number of ova shed (NOS) and estrous cycle (EC) of adult female rats with fourth day regular estrous cycles (REC), and we compared these effects with a MI of 100µg sulpiride solution in DMSO. Between 13:00-14:00h of different days of EC, CER groups received 20µL of pure DMSO into both OB; the sham groups received MI with 20µL distillated water (H_2O) into each OB. All rats with MI performed and an intact cyclic rat group were autopsied in the morning of next estrous vaginal shown (EVS). Both DMSO and H_2O MI does not modify the EC duration and NOS. Other groups of cyclic rats received a MI with 20µL sulpiride solution into each OB and were autopsied at next EVS. Just the sulpiride MI performed on diestrous-1 day delayed 24h the next EVS, but that don't occur in other sulpiride groups. The NOS were not modified in all experimental group. The DMSO direct administration in ovarian tissue does not affect gonadal primordial functions and its use is recommended like an infiltrating agent of non-polar drugs.

Spontaneous ovulation, Oestrous cycle, Dimethyl sulfoxide, Ovarian dopaminergic system, Sulpiride

Resumen

Se estudiaron los efectos de la microinyección (MI) dentro de las bursas ováricas (BO) del agente infiltrante dimetilsulfóxido (DMSO) sobre el número de ovocitos liberados (NOL) y el ciclo estral (CE) de ratas adultas con ciclo estral regular (CER) de 4 días y se compararon estos efectos de la MI con 100µg de sulpirida disuelto en DMSO. Entre las 13:00-14:00h de cada día del CE, grupos de animales con CER recibieron MI con 20µL de DMSO puro dentro de cada BO; los grupos testigo recibieron 20µL de agua destilada (H_2O). Todos los animales con MI y un grupo de animales cíclicos intactos se sacrificaron al estro vaginal observado (EVO). La MI del DMSO o H_2O no modificó la duración del CE ni el NOL. Otros grupos de animales con CER recibieron una MI con 20µL de sulpirida en cada BO y se sacrificaron al EVO. La MI con sulpirida en el diestro-1 retrasó el EVO 24 horas, pero no ocurrió en los otros grupos con sulpirida. No hubo cambios en el NOL entre los grupos. El DMSO administrado directamente sobre el tejido ovárico no afecta las funciones primordiales de la gónada y se recomienda su uso como agente infiltrante.

Ovulación espontánea, Ciclo estral, Dimetil sulfóxido, Sistema dopaminérgico del ovario, Sulpirida

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Introduction

Biological membranes are characterized by their extreme selectivity, so that several solutes cannot pass through them freely and even fail to interact with their lipids and proteins (Alberts et al. 2008).

Due to their polar properties, many experimental drugs and those in certified clinical use have difficulty in performing their actions at the membrane level, which forces the increase of doses and prolonged waiting times for these drugs to have the expected actions and effects (Brunton *et al*., 2012). On the other hand, it is known that the extracellular matrix plays a preponderant role in the mechanisms of recognition and biological immunity (Alberts *et al*., 2008). According to the above, how can the problem of rapid action of an active drug to solve a health problem be solved? The vehicle used as a means of transporting the drug can be a solution.

Dimethyl sulfoxide (DMSO; $(CH_3)2SO$) is a colorless liquid, has been used as an organic and industrial solvent, organ and tissue cryopreservative (Pegg, 2007), as a drug in Veterinary and Human Medicine (Parkin *et al*., 1997; Pope and Oliver, 1966) and, in the last twenty years, in the innovation of technologies in Molecular and Cell Biology (Chakrabarti and Schutt, 2001).

DMSO was discovered in 1866 by the Russian scientist Alexander Saytzeff. It is obtained as a by-product during the processing of wood pulp for paper manufacturing (Shirley *et al*., 1978).

The great potential of DMSO as an infiltration agent postulates it as an excellent choice for the experimental use of an array of active drugs whose water solubility is low. For example, dopamine antagonists are very insoluble in water and their use in in vivo systems makes it difficult to interpret experimental data when they are used as tools in the study of their effects on biological systems.
There is a growing interest in analyzing the functional role of DMSO for its amphipathic properties and its effects when interacting directly on the extracellular matrix in tissues where it is deposited as an infiltrative agent.

However, there is ambiguous and contradictory information on the supposedly toxic effects of DMSO, and therefore in the present work the biological effects in vivo of the solvent applied as an infiltrating agent in the ovarian tissue of the adult female rat were analyzed. Its direct effects when applied in living tissues were analyzed by looking for signs of cytotoxicity by means of conventional histological techniques of bright field microscopy and recording the duration of the estrous cycle, as well as the number of gametes released in the adult rat model, as indicators of the functional alteration of the gonad. On the other hand, its efficacy will be tested when used as an infiltrating agent of a specific and selective dopaminergic antagonist of the dopamine type 2 receptor, sulpiride.

Functional structure of the ovary

The ovaries of the rat are oval-shaped paired structures that vary in appearance and size depending on the stage of the reproductive cycle. The microscopic anatomy of the rodent ovary is shown in Figure 1.

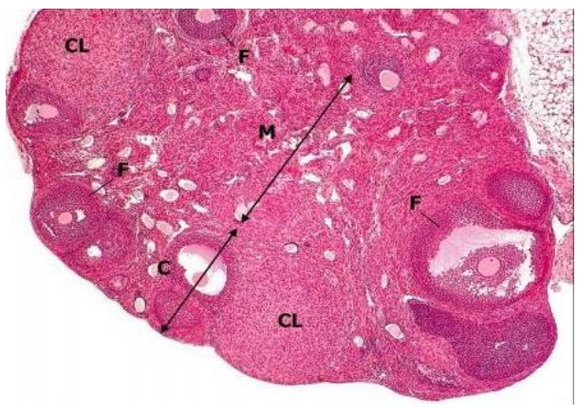

Figure 1 Microscopic anatomy of a normal rodent ovary (mouse, 40X). The cortex (C) contains numerous follicles (F) at various stages of maturation and corpora lutea (CL). The medulla (M), which is not always present in histological sections, contains lymphatic vessels, nerves and blood vessels
Source: (Taken from Cartwright and Moreland, 2008)

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Covering the surface of the ovary is a single layer of modified peritoneal mesothelium, the ovarian epithelium (OSE) and continues with the broad ligament (mesovarium) that provides support for the organ. The OSE of the ovary can vary in type from squamous to cuboidal, columnar to pseudostratified epithelium; this regional variation in the surface morphology of the OSE is accompanied with the cyclic changes that occur within the underlying ovarian parenchyma during the estrous cycle (Erickson, 1995; Figures 2 and 3).

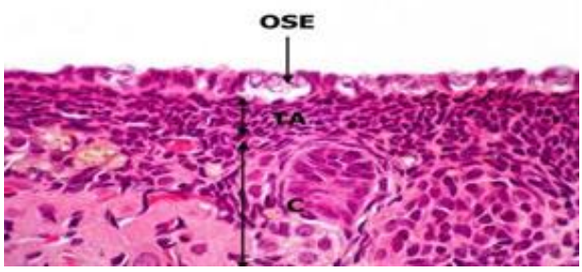

Figura 2 Ovarian superficial epithelium (OSE) - columnar type epithelium. Tunica albuginea (TA). Cortex (C) (rat, 400X)
Source: (Cartwright y Moreland, 2008)

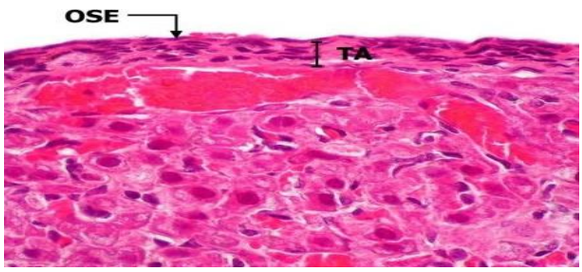

Figure 3 Ovarian surface epithelium (OSE) - squamous epithelium. Tunica albuginea (TA) (rat, 400X)
Source: (Cartwright and Moreland, 2008)

Three distinct zones can be distinguished in the mammalian ovary: 1) the cortex, which is the most dominant zone and contains the follicles at different stages of maturation and the corpora lutea, between the follicles are the supporting connective tissue and stromal cells. 2) the medulla, which contains a rich vascular network and connective tissue; and 3) the hilum, where the ovarian artery and vein, lymphatic vessels, nerve terminals and interstitial cells are found (Erickson, 1995; Sanchez-Criado, 1999; Yao and Barh, 1999) (Figure 1).

The ovary is lined by a single layer of epithelial cells: the germinal epithelium. The adult ovary consists of three different functional units: 1) the interstitial tissue, 2) the follicles and 3) the corpora lutea. These structures are in constant transformation. Thus, while some of them regress (e.g., atresic follicles become part of the stroma), others may form (the follicle after ovulation becomes a corpus luteum) (Erickson, 1995).

Future interstitial cells are found in the hilum, which are derived from mesenchymal cells in the stroma of the ovary. Interstitial cells synthesize and produce androgens which are very important in the regulation of a number of fundamental reproductive processes and are therefore of great physiological relevance (Erickson, 1995).

Four classes of interstitial cells are found in the ovarian stroma and are classified by their characteristics and position in the ovary as primary, thecal, secondary and hilar. The thecal interstitial cells originate from the stroma and migrate toward the basement membrane of the follicle where they are arranged in bands to form the inner and outer thecae. This migration toward the basement membrane takes place when the oocyte is maturing, and the follicle contains two or three layers of granulosa cells. After ovulation, the thecal cells are transformed into thecal luteal cells of the corpus luteum. The thecal-interstitial cells of the atretic follicles with antrum become part of the interstitial gland. In contrast, the cells surrounding the preantral follicles in which cell differentiation has not occurred and which enter atresia do not form part of the interstitial gland because they do not possess LH receptors. The same is true for thecal cells of preovulatory follicles that enter atresia and will not be part of the interstitial gland either. Interstitial thecal cells have receptors for LH, prolactin, adrenocorticotropin (ACTH), noradrenaline, GnRH and estrogens (Dominguez, 1993).

When the follicles fail to release their oocytes and consequently become atretic, the thecal interstitial cells are transformed into secondary interstitial cells that maintain steroidogenic activity and are innervated by adrenergic terminals. The interstitial cells synthesize and secrete testosterone in response to LH stimulation (Erickson, 1995).

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The ovarian follicle is the anatomical and functional unit of the ovary. Most follicles are located mainly in the periphery of the cortex, immediately below the tunica albuginea. The follicle consists of an oocyte, which is surrounded by granulosa cells that in turn form the corona radiata cells (cumulus oophorus), follicular antrum, granulosa cells, basal lamina, inner theca cells, theca-interstitial cells, connective tissue, outer theca (Greenwald and Terranova 1988; Erickson, 1995; Fawcett, 1995; Van Voorhis, 1999; Zhang, 1999) (Figure 4).

The corpus luteum is a transient endocrine gland, formed by follicular cells that remain in the ovarian tissue after ovulation (Juengel et al. 1999). The formation of the corpus luteum is initiated by a series of morphological and biochemical changes in the cells of the inner theca and granulosa membrane of the preovulatory follicle. This is called luteinization and occurs as a result of an increase in blood LH levels associated with the preovulatory surge of this hormone. A more complete description of the morphological changes associated with luteinization in the rat is that of Anderson and Little (1985), who state that after ovulation and follicular elimination, the follicle wall collapses and the granulosa cell layer is arranged in folds. The basal lamina that previously separated the granulosa cells and the inner theca is ruptured, where there is some extravasation of blood from the capillaries of the outer theca resulting in the formation of a central clot that invades the cavity of the ruptured follicle, the growth of these new vessels appears to be due to an angiogenic factor that must be secreted soon after rupture of the follicle (Figure 4).

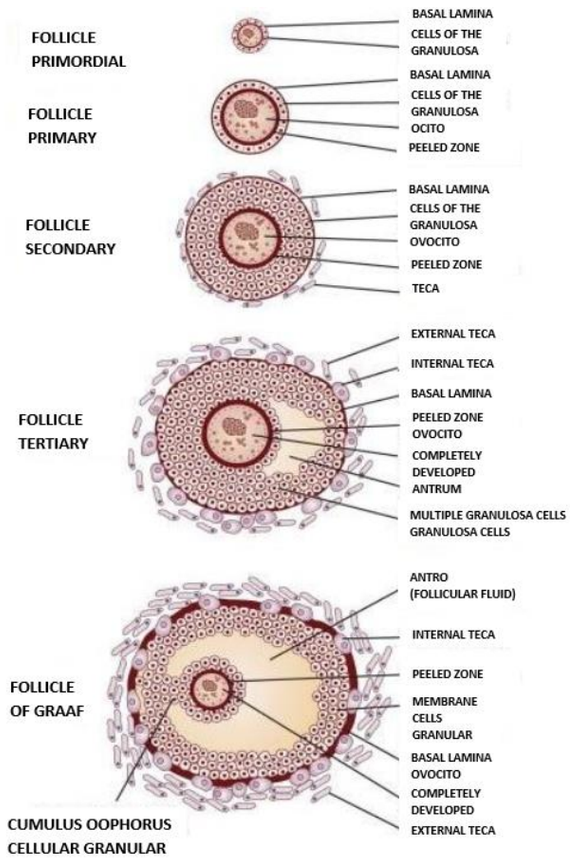

Figure 4 Diagram showing the development and different stages of ovarian follicle growth; throughout follicular development, the main compartments undergo gradual changes characterized by the proliferation of granulosa cells and theca cells, as well as the appearance of the antrum; At the onset of development, the oocyte leaves its prophase I dictyotene state and continues its differentiation until the completion of the second meiotic division if and only if it is fertilized by the spermatozoon
Source: (Taken from Bulun and Adashi, 2003)

Granulosa and inner theca cells undergo cytological changes. They enlarge and accumulate lipids and transform into pale polygonal cells, called luteal cells. In the rat, the number of junctional spaces between granulosa cells increases as the follicle matures, but decreases just before ovulation (Juengel et al. 1999; Niswender and Nett, 1988).

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The number of corpora lutea present in the ovary depends on the number of ovulations which, in turn, varies according to the species. After ovulation, the transformation of the follicle into a corpus luteum is mainly due to hypertrophy of the luteal cells and increased vascularization. Since the luteal cells are derived from the granulosa and inner theca cells, two types of luteal cells can be distinguished: those in the interior, which constitute the major part of the luteal tissue and are larger cells, and those in the periphery, which are smaller cells. Large luteal cells (granulosa-luteal cells, type II cells) are polyhedral and contain all the elements of steroid-secreting cells (mitochondria, smooth endoplasmic reticulum and secretory granules); on the contrary, small luteal cells are irregular and derived from theca cells (type I cell), contain ribosomes attached to the endoplasmic reticulum and do not present secretory granules (Niswender,1988).

Stral cycle

Much of what is known about spontaneous ovulation is based on knowledge of the estrous cycle of the rat. The rat cycle is unique in its brevity. The periodicity of light plays an important role in the length of the stages of the estrous cycle. The word estrus is a Latin adaptation of the Greek word oistros meaning: accessory, stinging or frenzy. This term was used by Heape, to describe the special period of the female's sexual desire and is distinguished from the male's cycle (Freeman, 1988).

The estrous cycle of the rat lasts 4 to 5 days and is regulated by endogenous factors, particularly by the interaction of the hypothalamic-pituitary-ovarian axis and can be influenced by exogenous factors such as light, temperature and chemicals sensed by the olfactory epithelium (Freeman, 1988; Kilen and Schwartz, 1999).

From the analysis of the changes of the vaginal epithelium, the estrous cycle is divided into four phases diestrous-1, diestrous-2, proestrus and estrus, below are the main changes of the vaginal epithelium in relation to the hormonal environment. The estrous cycle comprises a given period of time and is divided into the following phases: Estrus-1. This phase has a duration of 6 to 8 hours.

During diestrous-1 the plasma concentration of LH, FSH, estrogens and progesterone are basal. Follicles in all stages of growth are observed in the ovary. Mating and copulation are not allowed. Progesterone secretion by the corpus luteum and estradiol secretion by the follicles inhibit gonadotropin secretion. Vascularization and motility of the uterus are decreased. Vaginal smear shows leukocyte infiltration along with some cornified cells. Recently formed corpora lutea are the main source of progesterone secretion. During this day, functional regression of the corpus luteum begins as long as there has been no copulation, which in some rodents stimulates the release of prolactin (luteal-trophic hormone). Estradiol secretion by the growing follicles continues to increase (Freeman, 1988; Kilen and Schwartz, 1999).

Diestro-2. This phase lasts 55 to 57 h. Like the diestro-1 phase, ovarian steroids inhibit basal secretion of gonadotropins, which in turn maintain follicular growth. Plasma estrogen concentrations begin to increase in the afternoon of this day as a result of stimulation of the enzyme aromatase by FSH. FSH also stimulates mitotic division of the granulosa cells which results in the growth and differentiation of the follicles that will ovulate in this cycle. The corpus luteum continues to regress. The uterus is small, anemic and non-contractile (Freeman, 1988; Kilen and Schwartz, 1999).

Proestrus. The proestrus phase lasts 12 to 14 hours. The follicles have reached the stage of preovulatory follicles and secrete large amounts of estradiol. This hormone now exerts a stimulating effect (positive feedback) on the secretion of gonadotropins. On the morning of this day, plasma estrogen concentrations rise sharply, reach a maximum (preovulatory estrogen peak), and then fall sharply, which stimulates the activity of noradrenergic neurons in the hypothalamus, causing preovulatory GnRH secretion. This event stimulates preovulatory FSH and LH release, but FSH release occurs slightly earlier than preovulatory LH discharge. In the afternoon of the same day, maximum concentrations of these hormones are reached. The peak in plasma LH concentration stimulates the synthesis of plasminogen in the ovary, which initiates the cellular mechanisms that lead to the rupture of the follicle wall so that the oocyte can be expelled hours later.

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The interstitial gland secretes progesterone, which promotes ovulation. LH induces ovulation and luteinization of the remains of the follicle that released its oocyte. The uterus, by the action of estradiol, becomes extremely contractile and nucleated epithelial cells appear in the vagina. Copulation is accepted only in late proestrus, with the onset of the dark phase. FSH secretion on the morning of estrus, which is due to decreased ovarian secretion of inhibin, stimulates follicular growth (Freeman, 1988; Kilen and Schwartz, 1999; Sanchez-Criado, 1999).

Estrus. The estrous phase lasts from 25 to 27 hours. On this day, the estrus period occurs, in which the female shows patterns of sexual behavior. Approach, mating and copulation are accepted. As ovulation occurs in the early morning of this day, the eggs are in the oviduct. The postovulatory follicle begins to structure itself as a corpus luteum while a new series of primary follicles begin to develop. Numerous mitoses are found in the vaginal mucosa, displacing the more superficial layers (squamous and cornified epithelium), which are exfoliated into the lumen of the vagina. The presence of these cells in the vaginal smear is indicative of estrus and a sign of probable ovulation (Freeman, 1988; Kilen and Schwartz, 1999; Sanchez-Criado, 1999). The plasma concentration of progesterone increases on the day of estrus, while estrogens and LH are basal, except for FSH because in the morning of this day of the cycle a second peak in plasma FSH concentration occurs, although of lesser magnitude than in the afternoon of proestrus, whose role is to recruit the follicles that will ovulate in the following cycle (Freeman, 1988; Figure 5).

FSH binds to its respective receptor on the cell membrane and stimulates estrogen synthesis by the granulosa cells. The hormone-receptor complex acts on the adenylate cyclase enzyme system, induces an increase in cAMP and stimulates the synthesis and activity of the enzyme aromatase, which transforms androgens to estrogens (Freeman, 1988).

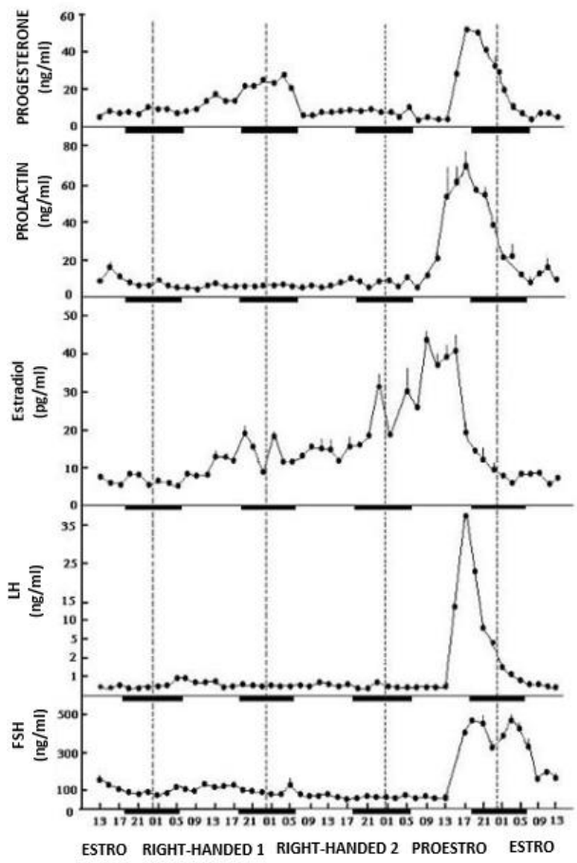

Figure 5 Plasma concentrations of progesterone, prolactin, estradiol, LH (Luteinizing Hormone) and FSH (Follicle Stimulating Hormone) obtained at two-hour intervals during the four days of the estrous cycle of the rat. Each point represents the mean ± E.E.M. concentration of the hormones. The black bars represent the dark phase from 18:00 to 06:00 hours the following day
Source: (Smith et al. 1975; cited by Freeman, 2006).

LH regulates estrogen synthesis by its effects on androgen production by thecal cells and by stimulating the synthesis and aromatase enzyme activity of granulosa cells that have previously been stimulated by FSH (Freeman, 1988). Prolactin inhibits the aromatase activity of granulosa cells and blocks the effects of FSH on the cells themselves resulting in decreased estrogen production. It also acts on the theca cells where it blocks androgen synthesis by inhibiting the formation of cAMP and enzymes that induce the cleavage of the cholesterol side chain.

Estrogen secretion by the follicle is regulated by FSH, LH, prolactin and other factors and hormones whose effects are coupled to those of FSH and LH. Some of these factors and hormones are: GnRH, oxytocin, epidermal growth factor, vasopressin, estrogens, adrenal corticosteroids and prostaglandin (Freeman, 1988).

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Hypothalamus-Pituitary-Ovary Axis

Of all the elements involved in the regulation of ovarian function, the hypothalamus is the site of control and integration of all the nervous and humoral signals coming from the CNS itself, the pituitary, the ovaries and the uterus. In contrast to other endocrine systems, the hypothalamic-pituitary-ovarian axis involves hypothalamic hormones (GnRH), various trophic adenohypophyseal hormones (LH, FSH, prolactin), ovarian hormones (estrogens, androgens and progesterone), peptide hormones and local factors and hormones produced by the uterus (prostaglandins, endogenous opioids and neurotransmitters). Gonadotropin secretion depends on the action of GnRH on the adenohypophysis (Fink, 1999). The output signal from the pituitary is, consequently, the pulsatile secretion of LH and FSH. This pituitary secretion is inhibited (negative feedback) by ovarian hormones (estradiol and progesterone) (Everett, 1988).

The ovary is a cyclic functioning gland, which implies that its components are ephemeral. The input signal is gonadotropins on the one hand, local factors on the other hand, and in some cases substances from the uterus (Speroff et al. 1999). FSH stimulates the growth and maturation of ovarian follicles, while LH causes follicle rupture under the influence of basal concentrations of FSH and LH, the granulosa cells secrete estrogens and at the time of follicular rupture undergo structural and biochemical transformations resulting in the formation of the corpus luteum (Niswender and Nett, 1988).

The output signal, independently of the mature ovum, includes ovarian steroids and inhibin which in addition to their peripheral actions regulate the secretion of GnRH, LH and FSH. While FSH acts only on granulosa cells, LH has multiple sites of action in the ovary: thecal, interstitial, granulosa and luteal cells.

FSH and LH are glycoproteins consisting of two different chains: α-subunit of 96 residues and β-subunit of 114 amino acid residues; and both subunits are linked by non-covalent bonds.

The α and β subunits are synthesized from precursors that undergo posttranslational maturation that includes the addition and modification of oligosaccharides; this glycosylation occurs in both the α and β subunits. It has been postulated that each stage of the maturation process represents a potential locus for physiological control of hormone formation and secretion (Counis, 1999; Fink, 1988).

Gonadotropins are synthesized in the adenohypophysis by specialized cells, the "gonadotropes". They are capable of producing one or several hormones. The synthesis and release of gonadotropins are stimulated by GnRH, a decapeptide secreted in a pulsatile manner by neurons located in different regions of the hypothalamus (Childs, 1999). Estrogens also stimulate the secretion of gonadotropins, particularly LH, through their effects on the pituitary gland and on neurons that regulate GnRH release (Fink, 1988; Blake, 1999; Weick et al. 1971).

Although GnRH stimulates the release of both gonadotropins, there is evidence that FSH and LH secretion is differential and involves other specific hypothalamic factors that stimulate and inhibit FSH secretion (Dominguez, 1993).

FSH and LH secretion is regulated by feedback mechanisms involving the CNS, the adenohypophysis and the gonads. In both the female and the male, basal secretion of gonadotropins is maintained throughout most of their reproductive life, however, in the female this basal secretion is interrupted by a massive release of gonadotropins that occurs periodically and precisely in the form of an intricate positive feedback cascade, which is induced and promoted by ovarian steroids. In both the female and the male, gonadotropin secretion occurs in a pulsatile manner. In the female, LH and FSH secretion is inhibited by estrogens (Fink, 1988; Aguilar, 1992).

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The secretion of gonadotropins is regulated by several neurotransmitter systems, the most studied being the catecholaminergic system (Vijayan and McCaan, 1978), in particular noradrenaline as a regulator of GnRH secretion and, therefore, of FSH and LH. From results obtained by pharmacological studies, it can be presumed that the participation of the various neurotransmitter systems in the regulation of gonadotropin secretion varies during the estrous cycle (Dominguez, 1993).

Like LH, FSH is released in response to GnRH stimulus and shows a maximal release when plasma concentrations of GnRH are increased in the portal-hypothalamic-pituitary system. In the rat, FSH release rates remain relatively high even after the drop in preovulatory LH secretion (Fink, 1988).

Preovulatory FSH and LH secretion is the result of two simultaneous events: 1) the increase in the frequency and amplitude of the secretory pulses of GnRH-ergic neurons in the portal-hypothalamic-pituitary system and, 2) the progressive increase in the sensitivity of the gonadotropes to this hormone (Childs, 1999; Fink, 1988).

The cascade of events leading to preovulatory LH release is initiated by increased secretion of 17 β-estradiol (Fink, 1988). Increased estradiol concentrations allow the expression of a neural signal for LH release: preovulatory GnRH secretion, as well as the progressive increase in the gonadotrope response to GnRH (Childs, 1999; Fink, 1988).

In the rat, preovulatory secretion of FSH occurs approximately 11 hours after that of LH. It has been suggested that this lag between the two preovulatory peaks may be the result of the following factors: that there is a hormone that specifically stimulates FSH secretion; that steroid hormones, inhibin or other factors differentially regulate the response of the gonadotropes to GnRH; or that LH secretion from the adenohypophysis is continuously exposed to GnRH, while FSH is triggered by but then independent of GnRH (Fink, 1988).

Another adenohypophyseal hormone important in the regulation of the estrous cycle is prolactin. Prolactin has a single polypeptide chain of 198 amino acids 198 amino acids, is synthesized as a prohormone and upon release undergoes proteolytic cleavage, thus forming the molecular structure normally found in plasma. Electrophoresis and chromatographic analyses of prolactin show that this hormone is not a single molecule, but a family composed of four molecular moieties, which are encoded by different DNA clones (Devesa and Tresguerres, 1992; Freeman, 1988; Nagy et al. 1999).

In the rat, estradiol stimulates prolactin release by acting directly on the adenohypophysis and indirectly by acting on target neurons in the brain (Freeman, 1988). Prolactin plays a fundamental role in the development of the mammary gland and the initiation and maintenance of lactation. It also modulates gonadotropin secretion from the anterior pituitary and thus gonadal function in both sexes (Devesa et al. 1992; Nagy et al. 1999; Weiner et al. 1988).

During the estrous cycle of the rat, plasma prolactin concentrations are low relative to those during the evening and night of proestrus, which correlates with the estrogen secretion profiles of the preovulatory follicles during this period (Freeman, 1988).

Prolactin is able to regulate GnRH synthesis and secretion. The control of prolactin secretion is established by a short feedback mechanism similar to what has been described for gonadotropins (Weiner et al. 1988).

Regulation of prolactin secretion is provided by two fundamental types of hypothalamic stimulatory (prolactin releasing factors or PRF's) and inhibitory (dopamine; prolactin inhibitory factors or PIF's) substances. Prolactin directly stimulates hypothalamic neurons producing PIF, which spills into the portal plexus and reaches the anterior pituitary, inhibiting its secretion. If prolactin levels decrease, PIF also decreases and prolactin biosynthesis and secretion by the pituitary is restored. The main PIF of prolactin secretion is dopamine (Freeman, 1988; Devesa et al. 1992; Nagy, 1999).

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As for FSH and LH, prolactin secretion occurs in a pulsatile manner, which depends on the mode of secretion of dopamine that acts as a PIF; this dopamine originates from the tuberoinfundibular system (A14) located in the medial basal hypothalamus. High-affinity dopamine stereospecific receptors have been detected in lactotropes and their density varies during the estrous cycle. Drugs that increase hypothalamic dopamine levels decrease circulating prolactin levels, as do drugs with dopamine agonist effect (e.g., bromocriptine) and conversely all dopamine antagonist drugs (e.g., haloperidol) produce an increase in circulating levels (Freeman, 1988; Devesa et al. 1992; Nagy et al. 1999).

Ovulation

One of the processes that culminates follicular growth and differentiation is the expulsion of the mature oocyte and is called ovulation (Domínguez, 1993).

Ovulation is considered to be the result of a localized inflammatory process, since before the oocyte is expelled there is edema in the internal theca and cell death and an increase in prostaglandins in the area. For ovulation to occur, several changes in the follicle wall and in the relationships between the granulosa and thecal cells are necessary. During the last stage of follicular growth and differentiation there is the disappearance of the desmosomes present in the granulosa and thecal cells, as well as the degradation of the collagen fibers, caused by fibrinolysin synthesized by the granulosa cells. This enzyme is activated by plasminogen, a product of the granulosa cells. The disappearance of desmosomes and granulosa cell junctions is a consequence of the decrease in estrogen concentration in the follicular liquor, produced immediately before ovulation, contains more water than that formed before and appears to be secreted at a higher rate since after the LH peak the capacity for estrogen synthesis by the granulosa cells decreases rapidly, while that of progesterone increases (Dominguez et al. 1991; Dominguez, 1993; Espey and Lipner, 1994).

Ovulation depends on a complex succession of endocrine phenomena involving the hypothalamus, pituitary and ovary. The central control of the cycle resides in the arcuate region of the basal medial hypothalamus. Neurons in this region have pulsatile GnRH release activity, which is transported by vessels of the portal-hypothalamic-pituitary system to the anterior lobe of the pituitary (Dominguez et al. 1991; Dominguez, 1993).

The regulation of ovulation is the result of a set of neuroendocrine events that modulate the growth and differentiation of ovarian follicles. These involve gonadotropins: (FSH) stimulates the development, maturation and differentiation of the ovarian follicles and (LH) causes the rupture of the mature follicle and the release of the oocyte (Fink, 1988). Also involved are hormones secreted by the ovary, particularly estrogens; hormones from the adrenal and thymus glands; trophic and energy-regulating hormones such as thyroid-secreted hormones and growth hormone; and classical and peptidergic neurotransmitters that reach the ovary via nerves or are synthesized in the ovary (Cruz et al. 1992; Dominguez et al. 1991; Espey and Lipner, 1994).

Background

Many drugs and various substances used as pharmacological tools in biological tests and bioassays that have proven physiological and physiotherapeutic activity present problems in their administration, particularly due to their insolubility. They frequently must be administered in an irritating or toxic vehicle. DMSO is an appropriate solvent for this class of water-insoluble agents and also presents biological properties not yet studied that could make it a suitable solvent for infiltrating drugs that act directly at the level of the extracellular matrix.

Why is DMSO, which is significantly less toxic and can be used as a replacement for other solvents, either pure or in mixtures of safe solvents, apparently underutilized? Perhaps the usefulness of DMSO has not been fully communicated to those in positions capable of making decisions about commercial solvent selection, or perhaps it is due to "biosafety myths" that have surrounded DMSO for several years (Borgioli, 2007; Vignes, 2000).

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Higher doses, more concentrated solutions, a higher frequency of exposure, or a longer exposure period may produce one or more of the toxic effects observed in some laboratory and clinical studies (Smith et al. 1983).

Tolerance limits for DMSO, however, have not been established for all exposure conditions. In addition, there are scientific papers and reports that show data recommending its therapeutic use in domestic animals and even in humans.

Physical and chemical properties of DMSO

DMSO is an amphipathic molecule with a highly polar domain and two apolar methyl groups, making it soluble in both aqueous and organic media (Santos et al. 2002).

DMSO dissolves various organic substances, including carbohydrates, polymers and peptides, as well as inorganic salts and gases. They generally do not have any serious biological effects, therefore, DMSO-water solutions of organic compounds can be used in various bioassays (Balakin et al. 2006).

DMSO is a polar, strongly hygroscopic solvent that shows solubility in water and lipids. Application of this substance to the skin results in hyperhydration of the stratum corneum with a subsequent increase in permeability. While it has no known effects on plasma membranes, it can cause changes in keratin filaments, its in vivo effects are short-lived (Brisson, 1974).

Most of the physiological properties of DMSO appear to be related to its penetration properties, its potential to inhibit or stimulate enzymes, and act as a free radical scavenger, among others. These properties are largely based on the chemical characteristics of DMSO, including its affinity for hydrogen bonds, affinity for water, ability to exchange water between cell membranes, and ability to react with organic molecules (Wexler et al. 2005).

Because of its property to rapidly cross the epidermis and cell membranes, DMSO has been proposed as an excellent carrier of drugs or poisons, which could represent multiple advantages for testing the effects of insoluble or hydrophobic drugs, in addition it has been reported that DMSO is less toxic than other amphipathic solvents used in the pharmaceutical and service industry, such as dimethylformamide, dimethylacetamide, N-methyl-2-pyrrolidone, among others (Baker, 1968).

Potential uses of DMSO in biology and experimental biomedicine

Numerous laboratory studies have documented the primary pharmacological actions of DMSO, including cell membrane penetration, effects on connective tissue, anti-inflammatory action, analgesia, diuresis, enhancement or reduction of the efficacy of other drugs, cholinesterase inhibition, vasodilatation, muscle relaxation, antagonism to platelet aggregation, among others (Jacob and Herschler, 1986).

DMSO is one of the most common solvents for in vivo administration of various water-insoluble substances. It is unclear how the response to a particular substance is altered by DMSO, but it is generally believed to act as a penetrant carrier of substances across membranes at all levels of biological organization.

The most important properties of DMSO as a penetrating carrier involve the ability to change the conformation of proteins and to replace water (Rammier and Zaffaroni, 1967).

DMSO is readily absorbed dermally and orally in animals and humans and enhances the absorption of many chemicals by these routes, DMSO at high concentrations is better absorbed than in dilutions in combination with water (Wexler et al. 2005). There are four main variables that influence the penetration of a solute through any membrane: 1) the diffusion coefficient across the membrane, 2) the concentration of the agent in the vehicle, 3) the partition coefficient between the membrane and the vehicle, and 4) the thickness of the membrane barrier.

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Penetrating agents are designed to affect one or more of these variables without causing permanent structural or chemical modification of the physiological barrier. Altering membrane thickness is less practical for drug delivery (it is difficult to conceive of non-toxic agents that could reversibly decrease stratum corneum thickness), so most penetrating agents, including DMSO, are believed to reversibly alter principles 1 through 3. There is some evidence to suggest that DMSO can increase diffusion through the stratum corneum by disrupting barrier function.

Some evidence suggests that DMSO may increase diffusion through the stratum corneum by disruption of barrier function, this likely occurs through aprotic interactions with intercellular lipids, as well as may include reversible distortion of lipid heads resulting in a more permeable packing arrangement.

In addition to the above, it is known to have an effect on less soluble agents in combination with a variety of vehicles as it favors increased penetration by transporting a higher concentration of the substance across the membrane barrier (Capriotti and Capriotti, 2012).

DMSO is commonly used in veterinary medicine as a salve, alone or in combination with other ingredients, in the latter case DMSO is used as a solvent to carry the other ingredients across the skin. In addition, in horses DMSO is used intravenously without combination or in combination with other drugs for the treatment of increased intracranial pressure and/or cerebral edema (Schleining and Reinertson, 2007a, 2007b).

Numerous studies have shown that DMSO increases skin permeability in humans and animals (Astley and Levine, 1976; Baker, 1968; Malten and Den Arend, 1978; Mitryukovskii, 1970; Scheuplein and Ross, 1970; Sweeney et al. 1966;). The concentration of DMSO determines the degree of change in permeability and solvent removal on partial or complete recovery (Astley and Levine, 1976).

DMSO has been used in various therapeutic situations in humans. In 1978 it was approved by the US Food and Drug Administration (FDA) for use in the treatment of interstitial cystitis by intravesical instillation (Parkin et al. 1997). Its effects do not appear to be related to a release of histamine from mast cells (Stout et al. 1995). It has been used successfully in dermatological (Burgess et al. 1998; Hsieh et al. 1987; Wong and Lin 1988;), urinary (McCammon et al. 1998), pulmonary (Iwasaki et al. 1994), rheumatoid and renal (Iwasaki et al. 1994) treatments. 1994), rheumatic and renal (Morassi et al. 1989), amyloidosis, (Salim, 1991; Salim, 1992a; Salim, 1992b; Salim, 1992c), DMSO crosses the blood-brain barrier (Broadwell et al. 1982) and has been effective in the treatment of traumatic brain edema (Ikeda and Long, 1990). It has also been used in the treatment of musculoskeletal disorders (Rosenstein et al. 1999; Zuckner et al. 1967) lung adenocarcinoma (Goto et al. 1996), rheumatological diseases (Abdullaeva and Shakimova, 1989; Murav'ev, 1986), chronic prostatitis (Shirley et al. 1978), dermatological diseases (Bertelli et al. 1995; Guerrey et al. 1999; Swanson, 1985) and as a topical analgesic (Kingery, 1997). Additionally, it has been suggested for the treatment of Alzheimer's disease (Regelson and Harkins, 1997).

Toxicology of DMSO

In addition to all of the above mentioned for pharmacological applications in the treatment of different pathologies, it is worth mentioning that several adverse systemic side effects have also been reported for the use of DMSO, such as nausea, vomiting (Davis et al. 1990), diarrhea (O'Donnell et al. 1981), hemolysis (Samoszuk et al. 1983), anaphylactic reactions manifesting with skin rash, flushing and occasionally bronchospasm (Berenson et al. 1987; Stroncek et al. 1991), renal failure (Smith et al. 1987), diastolic and systolic hypertension (Hameroff et al. 1983), bradycardia, heart block (Rapoport et al. 1991; Shlafer et al. 1976; Styler et al. 1992); rarely, pulmonary edema or cardiac arrest (Baum et al. 1992; Pegg and Kem, 1960).

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MORÁN-PERALES, José Luis, OLVERA-HERRERA, Jasiel Evani, HANDAL-SILVA, Anabella and GARCÍA-SUÁSTEGUI, Wendy Argelia. Direct infiltration effects of dimethyl sulfoxide into ovarian bursas on spontaneous ovulation and the estrous cycle of the rat: antagonism on the type 2 dopaminergic receptor. ECORFAN Journal-Ecuador. 2023

Other side effects of DMSO include: garlic-smelling breath and taste in the mouth due to pulmonary excretion of a small percentage of DMSO as dimethyl sulfide (Jacob and Herschler, 1983); its topical application, although well tolerated, may cause mild temporary local burning (Bertelli et al. 1995), skin rashes and pruritus (Swanson, 1985). A case of sulphohemoglobinemia has been reported after dermal application of DMSO in the treatment of interstitial cystitis with fatigue, cyanosis and dyspnea with mild exercise (Burgess et al. 1998).

The best documented side effect of DMSO treatment is intravascular hemolysis after intravenous infusion of 40% or greater solution that may result in urinary excretion of hemoglobin (Waller et al. 1983). Despite transient dose-dependent hemolysis and presenting hemoglobinuria, no alteration in renal function has been reported (Muther and Bennett, 1980).

Serum hyperosmolality has also been described in the control of increased intracranial pressure with intravenously administered DMSO (Wolf and Simon 1983); the same effect was also observed in in vitro studies in human blood (Santos et al. 2002); however, DMSO penetrates the cell membrane and causes an increase in osmolarity inside and outside the cell, preventing any significant hemolysis due to the formation of an osmotic gradient (Franco et al. 1983). One of the most important questions about any medicinal therapy is safety. Adverse reactions of DMSO are relatively mild and may occur in relation to its concentration and mode of administration (Jacob and De la Torre, 2009).

The acute toxicity of DMSO is low in animals. The LD50 in humans is 1800 mg/kg in skin and 606 mg/kg intravenously. The oral LD50 in the rat ranges from 14.5 to 28 g/kg and the dermal LD50 above 40 g/kg, intraperitoneal and intravenous LD50 in mice, rats and dogs exceeds 15 g/kg. It has been shown that acute lethal doses in experimental animals can produce tachypnea, restlessness, coma, hyperthermia, and sudden death, and can also cause death after several days due to renal failure (Jacob and Herschler. 1983).

DMSO is an experimental teratogen and also causes other reproductive effects in experimental animals (Wexler et al. 2005), however, so far there is no history that can explain the influence of DMSO in action as a teratogenic agent due to the diversity in the experimental procedures employed and the types of abnormalities that these studies indicate (Smith et al. 1983).

There is controversy about the apparent toxic role of DMSO in the rat, which is known to have teratogenic effects when administered during the first week of gestation inducing fetal abnormalities.

However, DMSO is not considered directly embryotoxic and has been shown to be a successful cryoprotectant for mammalian semen and embryos (Pegg, 2007).

Research has reported teratogenic effects in several species of experimental animals. Caujolle et al. (1967) observed developmental abnormalities including: malformations of limbs, beak, eyes and coelosomia in chicken embryos injected with a 50% DMSO solution. Other authors described anomalies such as exencephaly, microphthalmia, fused ribs and cleft lip in hamster embryos whose mothers were treated with an injection of 2.5 g/kg or more of DMSO on the 8th day of gestation (Ferm, 1966; Marín-Padilla, 1966; Staples and Pecharo, 1973;).

Both Caujolle et al. (1967) and Staples and Pecharo (1973) found evidence of abnormal embryos (anencephaly, malformed limbs, and celosomia) in mice injected intraperitoneally with 5 g/kg/day or more of DMSO during the second week of gestation. Juma and Staples (1967) reported increased resorptions in embryos of rats treated with 10.25 g/kg/day of 90% DMSO on days 8-10 of gestation, but no teratogenic effects. Caujolle (1967) observed an increase in developmental abnormalities when DMSO (5-10 g/kg) was administered daily from the 6th to the 12th day of gestation.

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Caujolle, (1967) documented that daily oral or subcutaneous doses of 4 to 5 g/kg of DMSO administered to rabbits on days 6 to 14 of gestation did not cause embryonic mortality or teratogenicity; and according to Staples and Pecharo, (1973) embryonic mortality increases with subcutaneous doses of 3 g/kg on days 8 to 11 but has no teratogenic effects. Several studies have been carried out with the aim of recognizing embryonic and fetal teratogenic abnormalities in rodents (Table 1).

Dose/ concentration	Route of administration	Effects	Reference
5-10 g/kg/d del 6º-12º día de gestación (50% DMSO)	Oral, Ip	Malformations of the nervous system, extremities, jaw, celosomia and edema.	Caujolle, 1967
10.25 g/kg/d from the 8th-10th day of gestation (90% DMSO)	Sc	Decreased number of live offspring, increased resorptions, no serious malformations.	Juma and Staples, 1967
10.25 g/kg/d from 8th-10th day of gestation (90% DMSO)	Ip	Not teratogenic.	Staples and Pecharo, 1973

Table 1 Teratogenic and embryotoxic effects in the rat
Source: (Smith et al. 1983)

Some teratological studies suggest that DMSO is not a teratogen in mammals when administered orally and dermally at doses that do not produce overt maternal toxicity, DMSO is not a teratogen at low doses, regardless of the route of administration.

Effects of the use of DMSO as a vehicle for teratogenic agents

The teratogenic effects caused by a single injection of the antimalarial agent pyrimethamine or the antitumor agent 6-mercaptopurine in rats on the 13th day of gestation were reduced by pretreatment with DMSO (Barilyak et al. 1978). However, in another study DMSO was not involved in the teratogenic action of pyrimethamine (Anderson and Morse, 1966). Injections of the insecticide dieldrin (days 6-14) into pregnant mice resulted in maternal and fetal toxicity which was increased when DMSO was used as a vehicle (Dix, 1977). Embryonic and fetal toxicity induced by the fungal metabolite (secalonic acid) was reduced when DMSO was used as a vehicle (Reddy et al. 1981).

Fetal mortality and abnormalities in hamsters treated with the insecticide thiram or disulfiram orally on the 7th or 8th day of gestation were increased when used together with DMSO (Robens, 1959).

In a study by Lauder and Salam (1972), seven substances with teratogenic capacity were evaluated in chick embryos to determine the effects that DMSO might have on development and mortality. The chemicals tested on four-day-old embryos were: 3-acetylpyridine, 6-aminonicotinamide, bidrin, sulfanilamide, 3-amino-1, 2, 4-triazole, physostigmine, and nicotine. DMSO had no effect on embryo mortality, however, there was an increase in teratogenic effects when used in conjunction with sulfanilamide, a decrease when used together with 3-acetylpyridine, 6-aminonicotinamide, 3-amino-1, 2, 4-triazole, and no change in combination with physostigmine and nicotine. Unfortunately, there are no precedents that can explain the influence of DMSO in action as a teratogenic agent because of the diversity in the experimental procedures employed and the types of abnormalities that these studies indicate (Smith et al. 1983).

Dopamine and dopaminergic receptors

Catecholamines are characterized by a catechol group: a benzene ring with two hydroxyl groups to which an amine group is attached. Catecholamines include dopamine (DA), norepinephrine (noradrenaline) and epinephrine (adrenaline). All catecholamines are synthesized from the amino acid L-Tyrosine. They are synthesized mainly in the brain, in the adrenal medulla and in some sympathetic nerve fibers (Tresguerres et al. 2008). Dopamine is synthesized by specific neurons that have only the first two enzymes of the biosynthesis pathway: tyrosine hydroxylase and L-Dopa-decarboxylase that act sequentially for the exclusive production of dopamine: dopaminergic neurons, which, after chemical or electrical stimulation, release dopamine at the synapse (García-Sevilla and Meana, 1988).

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Catecholamines play a key role in nutrient metabolism and body heat generation, stimulating not only oxygen consumption but also the consumption of fuels such as glucose and free fatty acids, thus generating heat. They stimulate glycogenolysis and the degradation of triglycerides to free fatty acids (lipolysis). They also play a role in regulating the secretion of multiple hormones. For example, as indicated above, dopamine inhibits prolactin secretion, but norepinephrine stimulates GnRH secretion and epinephrine inhibits insulin secretion by the beta cells of the islets of Langerhans of the pancreas (Garcia-Sevilla and Meana, 1988).

Dopamine is a neurotransmitter widely distributed in the central nervous system and some peripheral areas including the cardiovascular system and the renal system. In the brain, dopamine is involved in the control of movement, cognition, emotion, memory, reward and the mechanism of regulation of prolactin secretion by the pituitary gland. Several neurodegenerative diseases and psychiatric disorders have been linked to alterations in dopaminergic transmission (Hoffmann and Lefkowitz, 1996). The physiological effects of dopamine are mediated by membrane proteins that serve as receptors for specific chemical signals, termed dopaminergic receptors (DRRs), which have widespread expression throughout the mammalian brain (Rangel-Barajas et al. 2015).

According to Rangel-Barajas et al. (2015), DDRs belong to the G-protein-coupled receptor family. There are five mammalian receptor subtypes that are divided into two families according to their structure and biological response. The RDA1 family includes RDA1 and RDA5 receptors, the RDA2 family consists of RDA2, RDA3 and RDA4. RDA1 subtypes are positively coupled to adenylate cyclase (AC) to induce intracellular accumulation of 3,5 adenine monophosphatidyl cyclase (cAMP) and cAMP-dependent protein kinase (PKA) activation. In contrast, RDA2 is negatively coupled to CA, as a result of its activation, cAMP accumulation decreases and modulation of PKA activity and its effectors occurs. The activation of this receptor is also associated with other signaling pathways and may even act differently depending on the brain area or physiological conditions (Cooper et al. 1995).

Dopaminergic receptors and ovulation

Some years ago, the importance of the ovarian dopaminergic system has been demonstrated, confirming the observations of Dominguez and coworkers (1987). In that study, it was shown that in adult rats on day 1 of the estrous cycle, the dopaminergic receptor (DOR) system is indispensable for ovulation to occur on the expected day of estrus. The role of dopaminergic receptors in the rat ovary was recently reevaluated when three different dopaminergic antagonists: nonspecific antagonist haloperidol, type 2 receptor antagonist sulpiride (RDA2), and type 1 receptor antagonist SCH23390 (RDA1) were administered into the ovarian bursa at different times throughout the estrous cycle. Interestingly, sulpiride and haloperidol behaved similarly in "blocking" ovulation when administered on the night of estrus, all of estrus 1 and the morning of estrus-2, in contrast to SCH23390, which had effects only on the morning and evening of estrus-1. Increased tyrosine hydroxylase (TH) expression could also be shown in haloperidol administered animals, which could indicate an increase in dopamine synthesis, while expression of dopamine and cAMP-regulated phosphoprotein (DARPP-32) similarly increased, proteins that are activated by the interaction of dopamine and RDA1 (Venegas et al. 2015).

Dopamine has been found in the follicular fluid of human preovulatory follicles in high concentrations and in granulosa cell culture of human follicles has shown the presence of four of the five RDA subtypes, with the type 3 receptor being absent. In the rat ovary, type I and type II receptor mRNA was found in corpora lutea and interstitial tissue while in ovarian follicles its presence is moderate (Rey-Ares et al. 2007). The ovary also has the DA reuptake system, in human granulosa cells the dopamine transporter (DAT), catechol-O-methyltransferase (COMT), monoamine oxidase A and B, in addition to vesicular monoamine transporter 2 (VMAT2), while in Wistar and Sprague-Dawley rat strains it was shown that they express both DAT and VMAT (Greiner et al. 2008; Saller et al. 2014).

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Dopamine antagonists: sulpiride

Dopamine antagonists play an important role as a treatment against various psychiatric and neurological conditions, but also as a research tool, to understand the origin and development of these diseases or to create and improve therapies involving dopamine (Bahena-Trujillo et al. 2000).

Sulpiride: (RS)-N-[(1-ethylpyrrolidin-2-yl)-methyl]-2-methoxy-5-sulfamoylbenzamide, a neuroleptic and antipsychotic of the benzamine class, is used in the treatment of a wide range of psychotic disorders. It is a selective postsynaptic dopaminergic antagonist of RDA2 family receptors and does not produce extrapyramidal side effects like other benzamines in clinical use (Martin et al. 1996).

The effect of sulpiride occurs on limbic structures; it does not interact significantly with receptors of other neurotransmitters. It appears as an almost white crystalline powder, insoluble in water, slightly soluble in alcohol and methyl chloride and can be dissolved in mineral acids and alkaline hydroxides (Palomo, 1991).

Justification of the work

Due to the insolubility of dopamine antagonists and, in many cases, their low affinity to their receptors, this makes it difficult to use some dopamine antagonists in different bioassays, in this aspect DMSO can be very useful to infiltrate this type of poorly soluble agents. The biological effects of DMSO applied in the ovarian tissue of the adult female rat are unknown. Many drugs and pharmacological tools with proven physiological and physiotherapeutic activity present problems in their administration due to their insolubility and often must be administered in an irritating or toxic vehicle. DMSO is a solvent that has demonstrated great capacity to dissolve water-insoluble agents and also presents biological properties that have not yet been studied, which could make it a suitable solvent to infiltrate drugs that act directly at the level of the extracellular matrix.

For this reason, in the present work we analyzed the direct effects of DMSO when applied to live tissue, looking for signs of cytotoxicity by means of conventional histology with bright field microscopy, in addition to analyzing the effects that may alter the secretion of gonadotropins or affect ovulation, recording the duration of the estrous cycle, as well as the number of oocytes released in the adult rat model. On the other hand, its efficacy was tested by using it as a vehicle for a type 2 dopaminergic antagonist such as sulpiride, whose activity has been proven on these parameters, and comparing the effects of DMSO with similar studies in which other vehicles have been used for the administration of the antagonist, such as distilled water.

Methodology

Sixty-eight adult female rats of the CII-ZV strain, aged 90-120 days and with a body weight of 200-250 g, maintained under controlled lighting conditions (14 h light/10 h darkness; lights from 05:00 h and with free access to water and balanced feed. In order to monitor the phases of the reproductive cycle, the estrous cycle was recorded by means of vaginal smears taken daily between 09:00 and 10:00 h. Once the animals presented three consecutive four-day cycles (diestrous-1, diestrous-2, proestrus and estrus; cyclic rats) they were assigned to the different experimental groups. All experiments were in accordance with NOM-062-ZOO-1999.

Evaluation of DMSO cytotoxicity on spontaneous ovulation and estrous cycle length

Between 13:00-14:00 of Diestro-1, Diestro-2, Proestrus or Estro, groups of 6 cyclic animals were formed and sedated with isoflurane vapors and submitted to a dorso-bilateral laparotomy at the level of the inguinal region in order to exteriorize the ovaries and perform a microinjection inside each ovarian bursa with 20 μL of 100% DMSO.

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Similarly, the control groups received the microinjection with distilled water solution. In all animals, the recording of the estrous cycle was resumed the following day Animals were sedated with isoflurane vapors and sacrificed between 09:00 and 10:00h on the morning of the next vaginal estrus (observed vaginal estrus) the duration of the estrous cycle was recorded in each group, ovaries and oviducts were dissected where signs of ovulation were looked for and the number of released oocytes was counted. The ovaries of the animals were processed for brightfield histology, embedded in kerosene blocks, cut in microtome at 10 µm thickness (Gaviño et al. 1992) and stained with the hematoxylin-eosin technique (Luna, 1975). Signs of inflammation or necrosis in the ovarian tissues were recorded according to the methodology proposed by Yoshida et al (2009).

Analysis of the effect of specific dopamine receptor antagonism using DMSO as the infiltrating vehicle

Other groups of 8 and 4 cyclic animals respectively were distributed in experimental groups to perform the microinjection into the ovarian bursae of a dopaminergic receptor type 2 antagonist: sulpiride, using DMSO as a vehicle. Between 13:00-14:00 of diestrous-1, diestrous-2, proestrus and estrus, groups of cyclic animals were formed and sedated with isoflurane vapors and submitted to dorso-bilateral laparotomy at the level of the inguinal region to exteriorize the ovaries and perform a microinjection inside each ovarian bursa with 20 µL of sulpiride solution (5µg/µL) in 100% DMSO. As an absolute control group, 8 intact cyclic animals (Intact Control Group), sacrificed on the morning of vaginal estrus, were used. Similar to the previous experiment, in all these animals, the recording of the estrous cycle was resumed the following day The animals were sedated with isoflurane vapors and sacrificed between 09:00 and 10:00h on the morning of the next vaginal estrus (observed vaginal estrus) the duration of the estrous cycle was recorded in each group, the ovaries and oviducts were dissected where signs of ovulation were looked for and the number of oocytes released was counted. The ovaries of the animals were also processed for brightfield histology to record signs of inflammation or necrosis in the ovarian tissues.

Statistical analysis

The duration of the estrous cycle and the number of oocytes released were analyzed by Kruskall-Wallis test followed by Dunn's multiple comparisons test. Signs of tissue alteration or cytotoxicity in ovarian tissues were recorded in contingency tables and analyzed with Fisher's Exact Probability test, as appropriate. Differences equal to or less than $p<0.05$ were considered statistically significant.

Results

The body weight between the control groups and the groups treated with DMSO or sulpiride was similar (Intact Control (n=8): 218±5 grams vs Distilled Water (n=16): 247±4 grams vs DMSO (n=24): 235±4 grams vs Sulpiride (n=20): 235±4, ns). For such reason, ovarian and uterine weights were expressed in mg/100g body weight.

	H2O (n=4)		DMSO (n=6)		Sulpirida(n=4)	
	OI	OD	OI	OD	OI	OD
E	12.0±0.8	12.1±0.8	12.1±1.1	12.0±1.2	12.3±1.3	12.0±1.3
D1	10.2±0.3	10.4±0.5	12.0±0.7	12.4±0.8	11.1±0.6	12.3±0.6
D2	10.7±0.6	11.2±0.8	13.2±0.5	12.7±0.9	11.2±1.1	12.6±0.7
P	13.2±1.5	15.2±0.6	16.5±1.6	14.9±1.9	12.3±1.9	13.9±1.4
	Ovarian Mass					
	H2O (n=4)		DMSO (n=6)		Sulpirida (n=4)	
E	24.1±0.6		24.2±2.1		24.3±3.2	
D1	20.5±0.4		24.3±1.4		23.4±1.0	
D2	21.7±1.4		25.9±1.4		23.8±0.8	
P	28.4±1.6		31.4±3.4		26.3±3.2	
	Weight of the Uterus					
	H2O (n=4)		DMSO (n=6)		Sulpirida (n=4)	
E	187±16		168±18		175±29	
D1	173±10		181±21		187±16	
D2	163±4		179±10		191±29	
P	195±18		180±6		174±9	

Table 2 Relative weight of ovaries (mg/100 g body weight) ± s.e.m. and relative weight of uterus (mg/100 g body weight) ± s.e.m. in animals with regular four-day estrous cycle (cyclic) that received a 20 µL microinjection of: DMSO, Distilled Water (H2O) or 100 µg Sulpiride into the ovarian bursae at 13:00h on the day of Estrus (E), Diestrous-1 (D1), Diestrous 2 (D2) or Proestrus (P). The animals were sacrificed on the morning of the next observed vaginal estrus. LA: Left Ovary; RH: Right Ovary

No significant differences wer found in the weight of the ovaries or uterus between the different experimental groups (Ovarian mass):

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Intact Control (n=8): 26.3±1.6 mg vs Distilled Water (n=16): 23.3±0.8 mg vs DMSO (n=24): 26.5±1.2 mg vs Sulpiride (n=20): 24.2±0. 9, ns // Uterus: Intact Control (n=8): 163±7 mg vs Distilled Water (n=16): 180±7 mg vs DMSO (n=24): 177±7 mg vs Sulpiride (n=20): 183±10, ns) (Table 1).

Analysis of estrous cycle length between the groups treated with distilled water and DMSO did not show any alteration nor in the number of oocytes released (Table 2).

	Duration of the Stral Cycle		No. of Ocytes Released	
	H2O (n=4)	DMSO (n=6)	H2O (n=4)	DMSO (n=6)
Estro	4.0±0.0	4.0±0.0	13.5±0.9	12.2±1.4
Right-handed1	4.0±0.0	4.0±0.0	14.8±1.0	12.0±0.9
Diestro2	4.0±0.0	4.0±0.0	12.8±0.9	12.0±0.6
Proestrus	4.0±0.0	4.0±0.0	12.8±0.6	11.8±1.0
			13.4±0.4 (n=16)	12.0±0.5 (n=24)

Table 3 Duration of the estrous cycle (days)±e.e.m. and number of total oocytes released±e.e.m. in cyclic animals that received a microinjection of 20 µL DMSO or Distilled Water (H2O) into the ovarian bursae at 13:00h on one of the days of the estrous cycle. Animals were sacrificed on the morning of the next observed vaginal estrus

However, treatment with sulpiride induced a delay in the presence of vaginal estrus of almost 24 hours, but did not modify the number of oocytes released in this group with respect to the control group treated with DMSO or distilled water (Tables 3 and 4).

	Duration of the Stral Cycle DMSO + Sulpirida	No. of Ocytes Released DMSO + Sulpirida
Estro	4.0±0.0 (n=4)	11.5±0.5 (n=4)
Diestro-1	4.8±0.2* (n=8)	10.6±0.7 (n=8)
Diestro-2	4.0±0.0 (n=4)	11.0±0.4 (n=4)
Proestro	4.0±0.0 (n=4)	11.2±0.7 (n=4)
		11.0±0.3 (n=20)

Table 4 Duration of the estrous cycle (days)±e.i.m. and number of oocytes released±e.i.m. in cyclic animals that received a microinjection of 20 µL DMSO+100 µg sulpiride into the ovarian bursae at 13:00h on one of the days of the estrous cycle. The animals were sacrificed on the morning of the next observed vaginal estrus

	Distilled water (n=4)		DMSO (n=6)	
	NOL OI	NOL OD	NOL OI	NOL OD
Estro	7.3±0.5 (n=4)	6.3±0.9 (n=4)	6.0±0.7 (n=6)	6.0±1.2 (n=6)
Diestro-1	8.2±0.8 (n=4)	6.8±0.5 (n=4)	5.6±0.4 (n=6)	6.5±0.6 (n=6)
Diestro-2	6.3±0.5 (n=4)	6.5±0.5 (n=4)	5.8±0.3 (n=6)	6.5±0.5 (n=6)
Proestro	6.8±0.9 (n=4)	6.0±0.4 (n=4)	6.3±0.7 (n=6)	5.5±0.6 (n=6)
	DMSO + Sulpiride			
	OI		OD	
Estro	6.0±0.4 (n=4)		5.7±0.5 (n=4)	
Diestro-1	5.1±0.6 (n=8)		5.2±0.3 (n=8)	
Diestro-2	5.7±0.5 (n=4)		5.2±0.5 (n=4)	
Proestro	5.5±0.3 (n=4)		5.7±0.7 (n=4)	

Table 4 Number of oocytes released (NOL)±e.e.m. from the left ovary (LO) and right ovary (RO) of each of the cyclic groups of animals that received a microinjection of 20 µL of Distilled Water, DMSO or DMSO+100 µg sulpiride into each ovarian bursa at 13:00h of Estrus, Oestrus-1, Oestrus.2 or Proestrus. Animals were sacrificed on the morning of the next observed vaginal estrus

No significant alterations were observed in the histology of the main compartments of the ovarian cortex. The follicles and corpora lutea showed a normal appearance, with no signs of necrosis or other alteration compromising their function. Microinjection of DMSO showed some signs of vascular distention in the medullary zone of the ovary that are similar to the effects of microinjection with distilled water, 0.9% saline or 0.1% ascorbic acid solution (images of these last two vehicles were obtained from another similar study) (Figures 10 and 11).

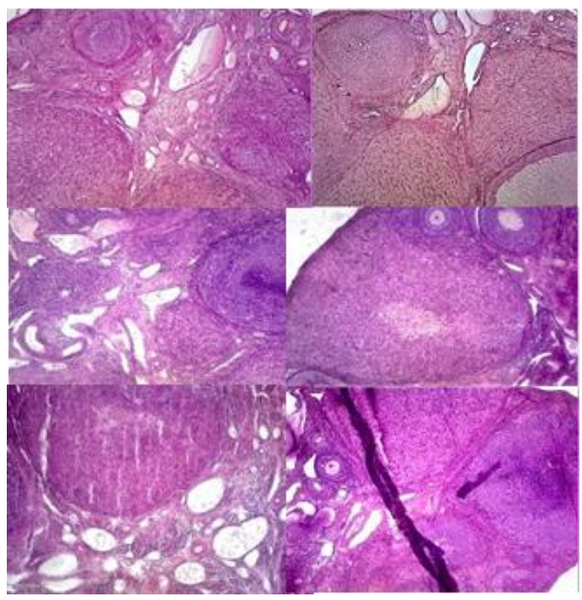

Figure 5 Images showing the effect of MI with 20 µL 100% DMSO; 20 µL SHAM (H2O) on the ovarian tissue of the rat that received treatment on the different days of the estrous cycle. Apparently, cortical tissues showed no signs of structural alteration; MO: ovarian medulla; VL: lymphatic vessel; A: arteriole; CL: corpus luteum; F: follicle; D1: diestrus 1; D2: diestrus 2; E: estrus.

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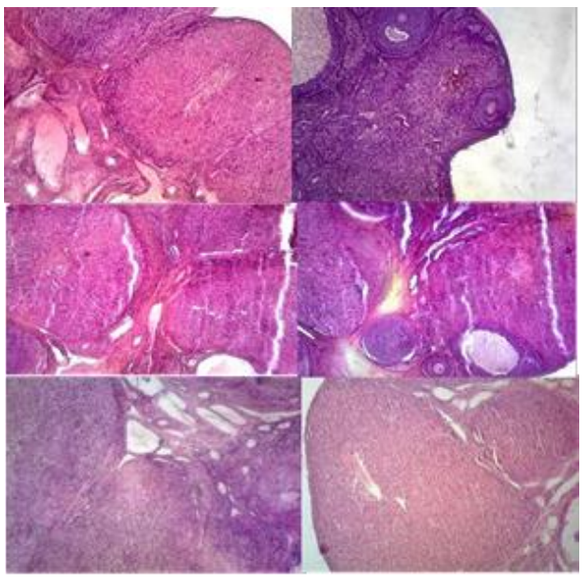

Figure 6 Images showing the effect of MI with 20 µL of 100% DMSO; 20 µL of 100% DMSO + sulpiride and 20 µL of Ascorbic Acid + sulpiride on the ovarian tissue of the rat that received treatment on the different days of the estrous cycle of the adult rat. Apparently, cortical tissues showed no signs of structural alteration; MO: ovarian medulla; VL: lymphatic vessel; A: arteriole; CL: corpus luteum; F: follicle; D1: diestrous 1; P: proestrus; E: estrus; S: sulpiride.

Discussion

The results of the present study show that DMSO did not induce significant changes in the cortical structure of the ovaries.

Apparently, infiltration of the solvent through the ovarian bursae did not have cytotoxic effects that compromised the primary functions of the ovary.

DMSO applied in therapies against inflammation (Dujovny et al. 1983; Parkin et al. 1997), viral (Aliaga et al. 1992) or bacterial (Pottz et al. 1967) analgesic (Gaspar et al. 2012; Kingery, 1997) and as a cryopreservative (Pegg, 2007) has shown fairly safe effects. Our results when applied directly to an organ within the body cavity show that its effects are easily controlled by the natural mechanisms of detoxification and elimination of waste substances, where the lymphatic system participates. As is known, the lymphatic systems comprise a one-way transport system for fluids and proteins by collecting them from the interstitial space and returning them to the blood circulation (Swartz, 2001).

It is a remarkable fact that the surgical technique and the application of DMSO administered directly to the ovarian tissue did not modify gonadotropin secretion or affect the primary functions of the gonads. That is, the rat organism rapidly readjusted to its substantive functions. The apparent non-alteration of ovarian functions is a reflection of the correct incidence of sex steroids in the higher centers controlling the secretion of GnRH and gonadotropins.

Based on the above, the use of DMSO as an infiltrative agent could be widely recommended to analyze the physiological or pharmacological role of experimental chemicals. In the present work, it was used as a vehicle for the infiltration of the dopaminergic antagonist sulpiride, and its use induced the same effects as those observed in other works when ascorbic acid 0.1% was used as a vehicle (Moonlighting). 0.1% as a vehicle (Letras Luna, 2016; Venegas et al., 2015; González-Quiroz, personal communication) and it is speculated that it would have similar effects to vehicles used to administer other dopamine antagonists, for example, alcohol-water mixtures, saline or distilled water (González et al. 2016.; Guzmán et al. 2018; Venegas et al. 2015; Venegas et al. 2017).

In order to use it as a harmless vehicle, it is clear that there is a lack of complementary studies that deepen the knowledge of the biosafety margin with which it can be used. For example, its effects could be compared with respect to other conventional vehicles supported with immunohistochemical techniques to analyze the presence of apoptosis indicators, expression of key proteins in the intracellular signaling cascades that lead to the biosynthesis of gonadotropin receptors, dopamine receptors, factors intimately linked to angiogenesis, to mention a few. Likewise, with simple molecular biology techniques, a comparative analysis of the eventual damage or alteration of the gene expression of these proteins could be carried out.

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There are multiple alternatives for scientific work in the field of Reproductive Biology and Experimental Reproductive Endocrinology where this infiltrating agent could be used. In our case, further analysis of ovarian functions and its organs (corpus luteum and ovarian follicle) or key tissues (theca cells, granulosa cells, oocyte or luteal cells), would require the use of an innocuous vehicle, which allows the solubilization of liposoluble substances and, most importantly, in minimum concentrations that produce clear physiological effects and not artificial ones. DMSO could be used as an effective vehicle to study the effects of bioactive drugs at lower doses and even shorter reaction times. According to novel information on the functions performed by the different anatomical-functional compartments of the ovary, it would be interesting to test its effects as an infiltrative agent and the production of reactive oxygen species and their indicators of oxidative stress, since these substances are known to be involved in ovulation, but also in various ovarian pathologies.

On the other hand, the effects of dopaminergic antagonism in ovarian tissue using DMSO as a vehicle reproduced previous findings on the alteration of neuroendocrine and endocrine signals leading to spontaneous ovulation. Thus, Venegas et al. (2015) studied the effects of microinjection of sulpiride and other dopamine antagonists through the ovarian bursae on spontaneous ovulation in rats with regular four-day estrous cycle and their results showed an estrous lag with a net delay of one day, which is consistent with our data, even compared to the hemicastered animal model (Letras et al. 2016).

The causes of vaginal estrus delay can be explained by alteration or disruption of sex steroids acting at the level of the central nervous system and hypothalamus. Somehow, in animals with dopaminergic receptor type 2 antagonism, the life of the corpus luteum is maintained twenty-four hours longer than expected, i.e., progesterone maintains its inhibitory effects on the centers leading to phasic discharge of GnRH and consequently, the preovulatory discharge of gonadotropins is withheld until the system accumulates enough estrogens to exert their stimulatory effects on the phasic secretion of GnRH and until that time, the ovary will be in a position to release oocytes when the preovulatory peaks of gonadotropins occur.

Venegas and collaborators (2015) demonstrated that GnRH injection in the afternoon of the expected proestrus induces ovulation in 100% in animals with dopaminergic antagonism, although in the model of the present study we did not verify this.

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Conclusions

- The results of the present study suggest that DMSO administered directly on ovarian tissue does not affect the main ovarian functions, estimated by the duration of the estrous cycle (indicator of gonadotropin and sex steroid secretion) or spontaneous ovulation (number of oocytes released).

- The administration of DMSO as a vehicle for sulpiride induced effects similar to those observed in other studies where other vehicles have been used; therefore, its use as an infiltrative agent would be advisable.

Perspectives

Based on the findings of the present study on the safety margin of DMSO as an infiltrative vehicle for the experimental use of dopamine antagonists, and perhaps other drugs, which due to their chemical nature are poorly soluble in aqueous solutions, further experimental trials are needed to promote their use.

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It is very likely that their use as a vehicle will facilitate handling, calculate and/or economize reagents, use biologically optimal doses to analyze the effect of chemical substances administered in living tissues or organisms, and prevent the installation of experimental artifices that could mask the true effects of pharmacological manipulations in experimental models.

In particular, our working group is interested in deepening the knowledge of the functional role that ovarian and hypothalamic dopamine plays both in the function of the gonad and at other levels of the hypothalamus-adenohypophysis-ovary axis. Therefore, it would be interesting to conduct an in-depth study on the acute or long-term effects that occur when DMSO comes into contact with the tissues of the ovarian cortex or in the brain of the rat, which form an essential part of the regulation of reproductive function. The function of dopamine in rat testicular tissue has also begun to be explored, since they have dopamine receptors and apparently play an important role in testicular function. In this context, the possible uses of DMSO as an infiltrating vehicle open a wide panorama for different studies in the field of Reproductive Neuroendocrinology.

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Article **ECORFAN Journal-Ecuador**
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Phytopathogenic fungi in seeds of the genus *Pinus*, stored in a gene bank

Hongos asociados a semilla del género *Pinus*, almacenada en banco de germoplasma

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Abstract

Phytopathogenic and saprophytic fungi present in seed with more than one year of storage were identified in the gene bank of the CONAFOR Jalisco delegation of the pine species: *Pinus douglasiana*, *P. devoniana*, *Pinus hartwegii*, *Pinus oocarpa* and *Pinus pseudostrobus*. The mycoflora present, its level of infestation and phytopathogenicity were identified. Physiological and sanitary quality was determined. The 5 species analyzed presented at least 4 genera of fungi: *Fusarium*, *Penicillium*, *Aspergillus* and *Rhyzopus* have been reported as storage fungi that cause seed deterioration. It is advisable to take measures to ensure sanitary quality, considering that the seed stored in the GDBs are the safeguard of forest genetic resources.

Phytopathogenic fungi in seeds, Seeds *Pinus* stored, *Pinus* genebank

Resumen

Se identificó la presencia de mico flora patógena presente en semilla con más de un año de almacenamiento en el banco de germoplasma de la CONAFOR delegación Jalisco de las especies de pinos: *Pinus douglasiana P. devoniana*, *Pinus hartwegii*, *Pinus oocarpa* Schiede y *Pinus pseudostrobus*. Con el objetivo de identificar la micoflora presente, su nivel de infestación y fitopatogenicidad. Se determinó la calidad fisiológica y sanitaria. las 5 especies analizadas, presentaron al menos 4 géneros de hongos: *Fusarium*, *Penicillium*, *Aspergillus* y *Rhyzopus* han sido reportados como hongos de almacén causantes de deterioro de semilla. es recomendable tomar medidas que aseguren la calidad sanitaria, considerando que la semilla almacenada en los BGF son el resguardo de los recursos genéticos forestales.

Hongos en semilla forestal, Semilla de *Pinus*, Bancos de germoplasma

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Introduction

Forest ecosystems provide products and services that contribute to human well-being, including food, timber, timber, medicinal plants, poles, firewood and fodder, among others, and environmental services such as the maintenance of water sources, biological diversity, climate regulation and carbon sequestration (Nordon, 2014).

Forest regeneration is the basis for species renewal and continuity; thus, production, dispersal, seed germination and recruitment are among the most important processes in the distribution and abundance of plant species (Nathan and Muller-Landau, 2000; Wang and Smith, 2002). Within temperate forests, the most abundant genera are oaks or Quercus spp. and pines Pinus spp. There are about 46 species of pines in Mexico (Sánchez-González, 2008); species widely used in their geographical distribution for their multiple uses.

Forest germplasm is a valuable and limited resource that is influenced by three factors: collection, management, and conservation, which are strongly related, as their effects are cumulative, with an increasing impact on seed quality (Vargas et al 2004). Since ageing is a natural and irreversible process, the higher the initial quality of the seeds and the better the storage conditions, the lower the rate of ageing and deterioration. In Mexico, the National Forestry Commission (CONAFOR) established the National Programme for the Management of Forest Genetic Resources in 2004, which sets out objectives and actions aimed at the conservation and sustainable management of forest genetic resources. Actions include the production of seeds of known origin or with genetic improvement (stands, seed areas and orchards) and germplasm banks (BGF). The BGFs have technical personnel and the necessary equipment to carry out the processes of collection, processing, storage and conservation of forest germplasm under controlled conditions of temperature and humidity, as well as the analysis of its physical and biological characteristics, with the purpose of conserving its germination potential. However, no analysis of sanitary quality or determination of fungal flora associated with the seed is carried out.

Fungi that lodge in seeds cause different types of damage; if the infection is very severe, the damage can lead to embryo death. With mild infections, seeds do not lose their germination power; however, their vigour may be affected. Fungi that have invaded seeds in the field become active again when the seeds are about to germinate, due to the high humidity present in the soil; some of these cause seed rot and seedling wilt. Many fungi do not cause problems to seeds and seedlings during germination and emergence, but are capable of causing the development of foliar, stem or fruit diseases, which reduce the quantity and quality of harvests (Moreno, 1995).

The objective of this work was to identify the diversity of pathogenic mycoflora present in seed with more than one year of storage in the germplasm bank of CONAFOR delegation Jalisco of the following pine species: Pinus douglasiana Martínez; Pinus devoniana Lindl.; Pinus hartwegii Lindl.; Pinus oocarpa Schiede ex Schltdl. and Pinus pseudostrobus Lindl. their level of infestation and phytopathogenicity.

Methodology

The physiological quality analyses were carried out in the seed laboratory of the germplasm bank of CONAFOR Jalisco state management, as well as in the seed laboratory of the University Centre of Biological and Agricultural Sciences (CUCBA) of the University of Guadalajara. Sanitary quality was analysed. Fungal quantification was performed by inducing mycelial growth and sporulation by incubation under the blotting paper or plotter protocol (Cimmyt, 2010) incubated at 27°C at 12-hour intervals of light and dark. Fungal identification was with the keys of Barnett and Hunter, 1998 and Cimmyt, 2010.

Results

The initial quality of the stored seed was low as the standard germination percentages obtained are within the range of 22 to 69 percent, taking into account that the seed was stored with a germination higher than 80%, it is considered with a considerable deterioration, Table 1.

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Species	Humidity (%)	Purity (%)	Feasibility (%)	Germination (%)
Pinus douglasiana	6.4	97.6	64	65
Pinus hartwegii	8	96.1	25	22
Pinus devoniana	6.9	99	64	54
Pinus oocarpa	11.3	89.2	66	69
Pinus pseusdutro	10.8	99.6	49	44

Table 1 Initial seed quality

In the 5 species analysed, at least 4 genera of fungi were present, of which: Fusarium sp., Penicillium sp., Aspergillus niger and Rhizopus, have been reported as storage fungi causing seed deterioration, generating abnormal seedlings and embryo death (Correa et al., 2012).

Species	Pathogenic flora encountered	% of infestation
Pinus douglasiana	*Aspergillus nigger*	18
	Penicillum	20
	Fusarium sp.	5
	Rhizopus ehrenb	12
Pinus hartwegii	*Aspergillus nigger*	40
	Chaetomium globosum	35
	Corynespora cassiicola	60
	Penicillium spp.	50
	Phoma westend	25
Pinus michoacana	*Aspergillus nigger*	20
	Chaetomium globosum	10
	Lasiodiplodia theobromae	5
	Penicillium	18
Pinus oocarpa	*Aspergillus nigger*	5
	Rhizopus ehrenb	4
	Corynespora cassiicola	12
	Phoma westend	8
Pinus pseudustrobus	*Fussarium moniliforme*	65
	Fusarium poae	48
	Lasiodiplodia theobromae	25
	Phoma westend	20
	Rhizopus ehrenb	15

Table 2 Fungal species identified on pine seeds

Fungi transmitted by conifer seeds can be classified into: saprophytes or weak pathogens; phytopathogens that infect and kill the seed embryo; pathogens of primary importance in seedlings; and phytopathogenic field fungi such as Fusarium spp. whose pathogenicity occurs in adult stages of the plant when conditions are conducive (Flores, 2010). Although most of these fungal genera are considered saprophytes, some of them do not always cause direct seed damage, but it is recognised that when the incidence is very high, seed vigour and viability tend to decrease (Mittal et al., 1990).

Although the management of seed in a germplasm bank ensures its conservation through low humidity and temperature, which prevents the development of microorganisms or pests, Campo-Arana et al. 2014, after analysing native forest seed from Colombia in germplasm banks, emphasise the importance of applying treatments to reduce the fungal load present, as well as the need to review the protocols for seed collection and storage.

Conclusions

Due to the incidence and pathogenicity of the fungal species found, as well as the loss of physiological quality of the seed, it is advisable to take measures to ensure its sanitary quality, especially considering that the seed stored in FGDBs are the safeguard of forest genetic resources.

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Article **ECORFAN Journal-Ecuador**
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Effect of inoculation with mycorrhizal fungi on the production of native corn (*Zea mays* L.) in Valle de Santiago, Gto.

Efecto la inoculación con hongos micorrícicos en la producción de maíz (*Zea mays* L.) nativo en Valle de Santiago, Gto.

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Abstract

The experiment was established from june-december 2022 in a randomised block design with three replications in experimental plot 1 of the Sustainable and Protected Agriculture Career of the Universidad Tecnológica del Suroeste de Guanajuato (UTSOE), located in the municipality of Valle de Santiago. The objective was to evaluate two varieties of native corn of the race harinoso de ocho (red and white) with inoculation with two commercial products containing mycorrhizal fungi (Rhizovibac® and Tec-myc®), a chemical fertilisation based on INIFAP (2015), of 80-40-00 and a fertilisation with vermicompost at a dose of approximately 100 g/mata. The experimental unit consisted of three furrows of eight metres long and 75 cm apart, where root volume - weight and yield were evaluated. Significant differences were found in Fisher's LSD ($p \leq 0.05$) for the variables of root volume and weight in the white maize 128.3 cm^3 above the mean 72.1 cm^3 with chemical fertilisation; however, the criollo corn was the one that obtained the best yield. It can be identified that there is a high relationship between root volume and root weight. In the case of the treatments of the commercial products used in the white corn with Tec-myc®, the lowest root volume - weight and the lowest yield was obtained with the use of Rhizovibac® in comparison with the red corn.

***Zea mays*, Mycorrhizal fungi, Eight-flour corn**

Resumen

El experimento se estableció de junio-diciembre del 2022 en un diseño de bloques al azar con tres repeticiones en la parcela experimental 1 de la Carrera de Agricultura Sustentable y Protegida de la Universidad Tecnológica del Suroeste de Guanajuato (UTSOE), ubicada en el municipio de Valle de Santiago. El objetivo fue evaluar dos variedades de maíz nativo de la raza harinoso de ocho (colorado y blanco) con la inoculación con dos productos comerciales que contienen hongos micorrícicos (Rhizovibac® y Tec-myc®), una fertilización química con base a INIFAP (2015), de 80-40-00 y una fertilización con lombricomposta a dosis de aproximadamente 100 g/mata. La unidad experimental estuvo conformada por tres surcos de ocho metros de largo y a 75 cm de distancia entre ellos, donde se evaluaron volumen - peso de raíz y el rendimiento. Se encontraron diferencias significativas LSD de Fisher ($p \leq 0.05$) para las variables de volumen y peso de raíz en el maíz blanco 128.3 cm^3 por arriba de la media 72.1 cm^3 con la fertilización química; sin embargo, el maíz criollo fue el que obtuvo el mejor rendimiento. Se logra identificar que existe una alta relación entre volumen y peso de raíz. En el caso de los tratamientos de los productos comerciales usados en el maíz blanco con Tec-myc® se obtuvo el menor volumen - peso de raíz y el rendimiento más bajo con el uso de Rhizovibac® en comparación del maíz colorado.

***Zea mays*, Hongos micorrícicos, Maíz harinoso de ocho**

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Introduction

Plants have developed numerous strategies since colonising terrestrial ecosystems to cope with various biotic and abiotic factors. One of the most effective is the ability of root systems to establish beneficial mutualistic symbiotic relationships with microorganisms (Camarena, 2012).

Mycorrhizal symbiosis in crops increases the absorption of nutrients and water, increasing yields by integrating its management with low and medium doses of fertilisers obtained through the inoculation of efficient strains, depending on the type of soil (Rivera *et al*., 2007).

The aim of this research was to evaluate the effect of the inoculation of two commercial products based on mycorrhizal fungi, as well as chemical fertilisation and organic fertilisation based on vermicompost, on two native maize varieties from the Bajío region of Guanajuato; the aim is to be able to recommend, especially to rainfed producers, a management alternative for this basic crop, which is currently affected by the lack of rainfall, or even if it does occur, it is concentrated in a narrow period of the agricultural cycle, affecting pollination and therefore yields, whether of grain or corn.

Literature review

Arbuscular mycorrhizae (AM) are ecologically mutualistic associations established between a select group of fungi (Glomeromycota) and the vast majority of plants. Approximately 80 % of the existing plant families have the potential to form this type of association (Trappe, 1987). AM are the type of mycorrhizae that form the majority of plants of agricultural interest. In such an association, the fungus forms arbuscules which are the structures where the exchange of carbon and phosphorus takes place between the fungus and the plant. Some mycorrhizal fungi form vesicles in the inner mycelium, which are reserve structures of the fungus.

The beneficial effects of AM are now well known and comprise increased uptake of poorly mobile elements in the soil such as phosphorus, copper and zinc by mycorrhizal plants compared to non-mycorrhizal plants (Smith and Read, 1997).

In addition, the more efficient use of soil nutrients by mycorrhizal plants saves chemical fertilisers and thus reduces pollution problems caused by excessive fertiliser use. There is also evidence that AM protect plants from pathogen attack (Newsham *et al*., 1995) and water deficit (Ruiz and Azcón, 1995).

Numerous field studies have demonstrated the benefits of mycorrhizal association in crops. Increased levels of root colonisation and hyphal density in the soil at early growth stages can increase phosphorus uptake and yield in maize (*Zea mays* L.), when the soil is deficient in this macronutrient (Deguchi *et al*., 2007).

Zulueta *et al*., (2021), found in a field study evaluating the co-inoculation of maize seeds with arbuscular mycorrhizal fungi and Azospirillum brasilense, that this microbiological interaction presented a higher percentage of mycorrhizal colonisation (62.7%), compared to the other treatments, among which chemical fertilisation reduced to 50% was also evaluated. Linked to the above, genetic richness has played an important role in Mexico, since native maize varieties are planted in regions, areas and ecological niches where improved varieties do not express their yield potential (Muñoz, 2003).

The Harinoso de Ocho race is characterised by its elongated cobs with a low number of rows and the development of large, mealy-textured kernels, with colouring ranging from pink to purple through purplish and red. It is mainly distributed in the west of the country, in Nayarit, Jalisco, Michoacán and Guanajuato (CONABIO, 2011).

Soft floury maize has white glazed kernels, which are considered to be derived from the race Harinoso de Ocho with introgression of Chapalote or Reventador (Wellhuasen *et al*. 1951).

Materials and methods

Location of the experiment

The experiment was established from July to December 2022, in the experimental plot 1 of the Sustainable and Protected Agriculture course of the Technological University of Southwest Guanajuato, in an area of approximately 500 m^2, under rainfed conditions, at the coordinates 20° 24' 01.5" N and 101° 13' 19.5" W.

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Planting material and planting density

Two varieties of native maize of the race Harinoso de Ocho, both red or "colorado" and white, which are widely used in the Valle de Santiago region to make pozole, were available. Both varieties were sown manually at the bottom of the furrow, on July 1, depositing two seeds per coup or clump, at a distance between clumps of 50 cm and in furrows 75 cm apart, to work a density of 53,000 plants per hectare, all under rainfed conditions.

Treatments and experimental design

The treatments (T) consisted of by the combination of these two varieties of native maize, with inoculation with two commercial products with mycorrhizal fungi (Rhizovibac® and Tec-myc®), a chemical fertilisation based on INIFAP (2015), of 80-40-00 and a fertilisation with vermicompost at a dose of approximately 100 g/mata, being conformed as follows: T1= white maize with chemical fertilisation; T2= white maize with vermicompost; T3= white maize with Tec-myc; T4= white maize with Rhizovibac; T5= red maize with chemical fertilisation; T6= red maize with vermicompost; T7= red maize with Tec-myc and T8= red maize with Rhizovibac. In this way, a factorial experiment was carried out with eight treatments, under a randomised complete block experimental design with three replications. The experimental unit consisted of three furrows of eight metres long and 75 cm apart, with the central furrow as the useful area.

Variables evaluated

Root volume: this was measured with the help of a graduated container, gauged with water, measuring and averaging the volume of water dislodged by three roots at the moment of submerging each one separately, previously washed to remove the water. Each one separately, previously washed to remove the soil. It was expressed in cm^3.

Root weight: measured with the aid of a digital balance, weighing and averaging three roots per replicate, previously washed to remove the soil. It was expressed in grams.

Yield: It was obtained at the end of the crop cycle, harvesting the cobs in six linear metres of the central furrow (4.5 m^2). They were shelled and the grain was weighed. It was reported in kg.

Agronomic management

During the crop cycle, two manual weeding, two applications of garlic extract for thrips control, one foliar application of Bacillus thuringiensis for Spodoptera frugiperda control and one application of foliar fertiliser at the flag leaf stage, prior to flowering, were carried out.

Data analysis

Data were analysed by analysis of variance (ANOVA) and Fisher's LSD test ($p \leq 0.05$) using Fisher's LSD test package ($p \leq 0.05$). Using the statistical package Minitab 18®.

Results and discussion

The analysis of variance showed significant differences for the variables evaluated (root volume, root weight and yield).

The LSD Fisher mean comparison test ($p \leq 0.05$) in Table 1, for treatment T1 and T8 are the treatments whose root volume and root weight were higher than the overall mean. The difference is that T1 is the treatment with chemical fertilisation and T8 is the treatment with chemical fertilisation.

Rhizovibac. This is in agreement with Tadeo et al (2017), where the use of varieties, as well as fertilisers and mycorrhizal fungi allow complementing the use of chemical fertilisers, which could increase production. It is known that, under conditions of nutrient scarcity, plants have the ability to modulate the architecture and functionality of their root system, potentially increasing nutrient uptake (Lynch, 1995). Nutrients are distributed heterogeneously in the soil and plants respond to the local concentration, allocating a higher nutrient production to their roots (Lynch, 1995). Concentration, allocating greater root production to regions of higher availability (Grossman and Rice, 2012). Possibly what happened in this experiment concentration, allocating greater root production to regions of higher availability (Grossman and Rice, 2012).

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Possibly what happened in this experiment maize is related to the diversity of mycorrhizal fungi. The correlation coefficient test (table 2) was carried out for the variables evaluated. It can be seen that there is a high correlation between fresh weight and root volume.

Treatment	Root volume (cm^3)	Root weight (g)	performance (kg)
T1= maize White with chemical fertilisation	128.3 a	108.33 a	0.576 ab
T2= maize White with lombricomposta	33.33 b	53.00 ab	0.525 ab
T3= maize White with Tec- myc	25.833 b	45.667 b	0.513 ab
T4= maize White with Rhizovibac	77.5 ab	99.00 ab	0.358 b
T5= maize red maize with chemical fertilisation	53.33 ab	92.66 ab	0.595 a
T6= maize red maize withlombricomposta	83.33 ab	84.00 ab	0.435 ab
T7= maize red maize withTec-myc	43.33ab	52.00 ab	0.435 ab
T8= maize red maize with Rhizovibac	131.7a	102.667 ab	0.416 ab
Media	72.1	79.67	0.4842
LSD	44.1	27.3	0.104
Fisher's LSD (least significant difference) ($\alpha \leq 0.05$). Means that do not share a letter are significantly different.			

Table 1 Comparison of means for variables measured in the mycorrhizal fungal inoculation experiment on native maize production in Valle de Santiago,Gto.

On the other hand, the T5 treatment consisting of red maize with chemical fertilisation is the one that obtained the best yield, which suggests that mycorrhizal fungi are complementary in agricultural production. Despite their influence on plants to adapt to nutrient deficient soils by facilitating their adaptation, this may affect growth characteristics as in the case of T8.

In this experiment it was expected that maize would have the same response to the use of mycorrhizal fungi (table 1), but there are differences, possibly due to a specificity of mycorrhizal fungi to the type of maize used as observed in the work of (Sangabriel-Conde et al., 2015).

Where, the different mycorrhizal species colonised only 2-4 types of maize, suggesting that the variety of mycorrhizae colonised only 2-4 types of maize, suggesting that the variety of mycorrhizae colonised only 2-4 types of maize.

	Volume	Fresh weight
Fresh weight	0.824**	
Performance	0.07	-0.103
** Highly correlated		

Table 2 Pearson's correlation of variables measured in the inoculation experiment with mycorrhizal fungi inoculation experiment with mycorrhizal fungi in the production of native maize in Valle de Santiago, Gto.

Although plants rely on their roots to acquire nutrients, they form mutualistic symbioses with arbuscule mycorrhizal fungi regularly belonging to the phylum Glomeromycota (Smith and Read, 2008). With respect to direct nutrient delivery, mycorrhizal fungi benefit the host plant by promoting root growth (Ramírez-Flores et al., 2019), such is the case in treatment T8. Thus, plant productivity is often limited by nutrient availability. Under conditions of nutrient scarcity, plants have the ability to modulate the architecture and functionality of their root system, even when associated with the right type of mycorrhizal fungi.

Conclusions

The main treatment for most of the variables evaluated in this research was treatment T1 white maize with fertilisation 80-40-00 fertilisation, which favours root growth and weight, and in the case of red maize (T5) the best yield was obtained. On the other hand, the use of Rhizovibac® was a better result in red maize, producing 131.7 cm3 above the average of 72.1 cm3. By increasing the root volume in a plant, it is more likely to cope with biotic and abiotic changes that may occur inside the rhizosphere, so it is suggested that the use or implementation of mycorrhizal fungi is a complement to sustainable agricultural production.

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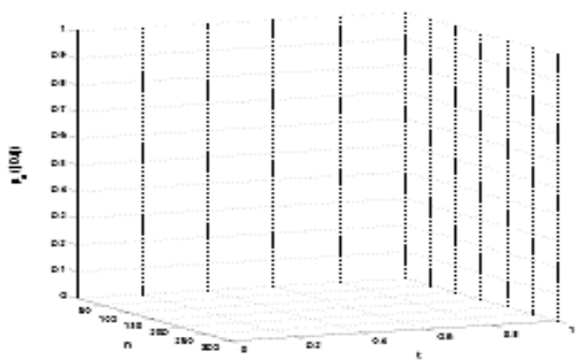

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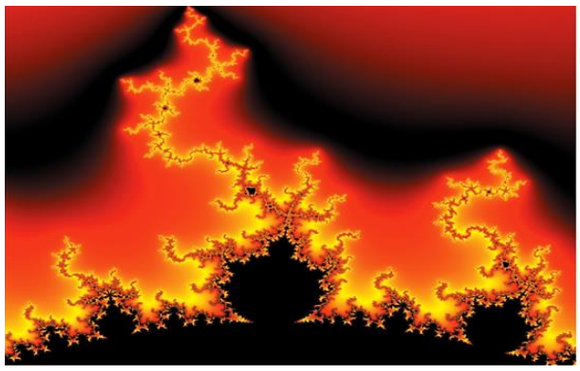

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