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Changes in the Mare Oviduct Across Different Seasons Throughout the Year

Noelia González[1] | Agatha Varela[2] | Nacho de Blas[1] | Lydia Gil[1]

[1]Department of Animal Pathology, Faculty of Veterinary Sciences, Instituto Universitario de Investigación Mixto Agroalimentario de Aragón (IA2), Universidad de Zaragoza, Zaragoza, Spain | [2]Hospital Veterinario La Chopera. P.° de la Chopera, Madrid, Spain

Correspondence: Noelia González (noegorti@unizar.es)

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ABSTRACT

At present, oviductal pathologies and their influence on mare fertility have not been clearly determined. Checking changes throughout the year in the oviductal structure may be relevant to understand the influence on the appearance of problems in oviductal functionality and, thus, the impact on fertility, which is the objective of this work. The oviducts of 56 mares were examined. Their anatomy, permeability, oviductal cellularity and the presence of pathologies were assessed to establish the correlations between the time of the year and each of these parameters. We cannot conclude whether there is a correlation between oviductal permeability or the presence of pathologies and season. Also, we did not find a relationship between oviductal permeability and the presence of pathologies; in fact, most of the oviducts analysed were permeable throughout the year (88.13%), and only 5.93% showed partial permeability, while 5.93% were not permeable. Regarding pathologies, 7.62% of the oviducts presented adhesions, with an increased incidence during anoestrus and the spring transitional period, while 23.72% of the oviducts presented paraoviductal cysts and 41.52% presented paraovarian cysts. The incidence of oviductal thickening was observed only during the reproductive season (14.7%). Regarding oviductal cellularity, it´s correlated (0.236) with the ovarian activity observed throughout the year.

1 | Introduction

The oviduct is a tubular organ that connects the ovary to the uterus (Aguilar et al. 2012). The mare's oviduct is the longest among domestic mammals, reaching 20 cm and sometimes even 30 cm (Getty 1982). In the oviduct, fundamental phenomena of reproduction take place, including the transport of male and female gametes, the maintenance of the sperm reserve, the completion of sperm capacitation and fertilisation, and the first divisions of the embryo while it is transported to the uterus (Goudet 2011).

Anatomically, we can distinguish three parts (Figure 1): infundibulum, ampulla and isthmus. This differentiation is based on the anatomical and histological characteristics of each area (Mouguelar et al. 2015). In a mare, the uterine orifice of the oviduct presents a papilla that is a barrier against ascending infections (Konig and Liebich 2011). The papilla also prevents the migration of unfertilised oocytes into the uterus (Senger 2011). This structure exerts a protective function, decreasing the incidence of ascending infections associated with infertility. In addition to infections, other oviductal pathologies can appear that imply subfertility or infertility problems (Allen et al. 2006), including the presence of gelatinous masses, which can pose a problem for the transport of gametes or even the embryo itself. Paraovarian, intraepithelial and infundibular cysts are some common pathologies (Figure 7).

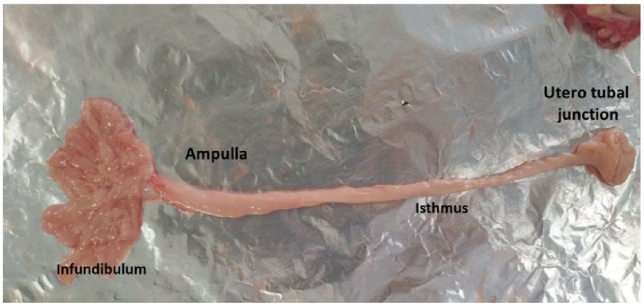

FIGURE 1 | Oviduct anatomy.

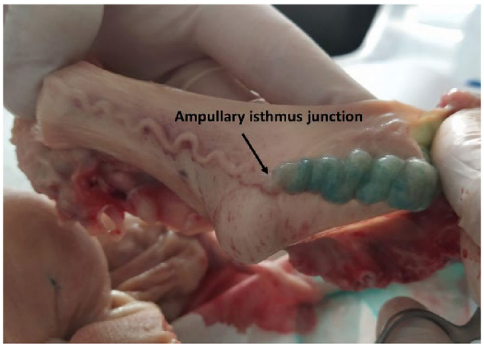

FIGURE 2 | Detail of the ampulo- isthmic junction.

When observing the anatomy of the oviduct, we can see that, on its upper edge, the infundibulum has irregular fimbriae, which are partly attached to the cranial pole of the ovary and form the cranial edge of the ovulation fossa. The rest of the fimbriae and the infundibulum extends over the ventral edge of the ovary and covers the ovulation fossa (Samper 2009), giving it a funnel shape. The oviduct is contained in the mesosalpinx, a peritoneal fold of the lateral layer of the broad ligament that forms a ventrally opening pocket on the lateral surface of the ovary (Kenney 2010). The ampulla contains multiple branched epithelial folds of different complexity in all its histological sections and during all reproductive phases (Kainer 1993). The ampulo-isthmic junction (Figure 2) has cryptic structures with a glandular appearance at the base of its primary folds. The luminal area and the epithelial perimeter of this segment are greater than those of the isthmus, which is defined as the region with the smallest diameter and, in turn, as the longest segment of the oviduct (Mouguelar et al. 2015). Its total diameter is around 2–3 mm, and it has a thick muscular layer, which contains short mucosal folds (Kenney 2010).

Histologically, the oviduct consists of three concentric layers: the outermost layer or serous layer, which is composed of mesothelial and nonstriated muscle cells derived from the broad uterine ligament; the middle layer or muscular layer (myosalpinx), which is organised differently depending on the species (Muglia and Motta 2001); and the inner layer, or mucosal layer (endosalpinx), which has a dense lymphatic and vascular supply, with adrenergic, cholinergic and peptidergic nerve fibres (Wrobel et al. 1993). The mucosa is lined by a simple columnar or pseudostratified epithelium containing ciliated and secretory cells (Teilmann et al. 2006, Kölle et al. 2009). Ciliated cells facilitate the transport of eggs, sperms, and embryos along the oviduct, in collaboration with Cajal interstitial cells present in the muscle (Dixon et al. 2009). Secretory cells have a rough endoplasmic reticulum, a highly developed Golgi apparatus, and usually apical electro-dense granules with or without microvilli. Their role is to create an optimal microenvironment for the functions that will develop in the oviduct (Hugentobler et al. 2010, Killian 2011). Ciliated cells are the predominant cells in the oviductal tract, although they are not evenly distributed; the segment with the least number of ciliated cells is the isthmus, and the segment that contains the highest number is the ampulla. Non-ciliated cells, characterised by having apical protrusions with microvilli, are also present throughout the oviduct, although with a greater presence in the ampulla and progressively decreasing toward the isthmus (Desantis et al. 2011). Ciliated cells have a round nucleus, while non-ciliated cells have an elongated nucleus with an irregular contour and an evident nucleolus. Depending on the types of cells and the oviduct section in which they are located, the density of their cytoplasm differs (Desantis et al. 2011). Also, some particularities have been described in the literature regarding the equine oviduct, such as the presence of connective trabeculated tissue dividing the lumen (Aguilar et al. 2012), the presence of glanduliform structures in the mucosa (Aguilar and Reyley 2005) or the presence of cell–collagen masses (Aguilar et al. 1997, Lantz et al. 1998).

In fact, regardless of the time of the year, the temporarily covered of the oviduct by the accumulation of naturally occurring collagenous or globular oviductal masses (Allen et al. 2006, Liu et al. 1990) could be the reason for failing to become pregnant when mated or inseminated with fertile semen in the absence of identifiable pathologies in the structures and functions of their reproductive system. These globular masses are commonly found in the oviduct of fertile mares, at rates between 42% and 88% (Tsutsumi et al. 1979, Saltie et al. 1986, Aguilar et al. 1997, Arnold and Love 2013), and they tend to be present in the ampulla near its junction with the isthmus (Kenney 2010). In a study conducted by Medenbachl et al. (1999), it was found that half of the mares examined had eosinophilic masses. Are masses of variable size, usually unilaterally and located near the ampulo-isthmic junction and consist of swirling bundles of fibres. The masses contain neutral mucopolysaccharides (Onuma and Ohnami 1975), type I collagen (Liu et al. 1990) and degenerated cells, and are not connected to the epithelium and frequently distend the lumen.

Adherences are another pathology that could be present in the oviduct and affect fertility. In a study conducted by Medenbachl et al. (1999), approximately 50% of the mares examined had thin, mostly bilateral, connective tissue fibres linking the ovary to the oviductal fimbriae that project into the oviduct. In no case did they observe that these adhesions blocked the infundibulum. However, in a study by Pye et al. (2018) that examined mares that underwent laparoscopic PgE_2 gel application and had one or more perpendicular adhesions, 69% of the mares whose reproductive histories showed failures in conception without apparent reproductive pathology, it was evidenced that they had adhesions that ran transversely through the oviduct during surgery.

The presence of cysts in the oviduct could not be forgotten, because they are relatively common. In a study conducted in 1999, Medenbachl et al. (1999) showed that 66.6% females had a marked presence of them, usually presented bilaterally. These cysts were frequently found in the ampulla, but not in the isthmus, and they could only be categorised histologically. They were found to be usually located in the epithelium or at the base of the mucosal folds. Their diameter varied from 10 to 100 micrometres. In the other side, paraovarian cysts are more easily identifiable, and they are the most common type of cysts found in the mare ovary, being small and multiple. These cysts are usually found in the fimbriated portion of the oviduct and, in principle, do not interfere with fertility. They are usually remnants of the mesonephric and paramesonephric tubules or ducts, as well as the oviductal ones (Blue 1985).

Last, oviduct inflammation or salpingitis may be another relevant pathology. While this pathology is clearly described in sows (Wilson et al. 1949) and cows (McEntee 1990), its presence is not usual in equine species (Medenbachl et al. 1999). In mares, it usually occurs because of a non-occlusive, infiltrative and occasionally exudative process characterised by diffuse or focal, marked lymphocytic infiltration into the epithelium and *Lamina propria* (Vandeplassche and Henry 1977). Lymphocytic infiltration in the ampulo-infundibular region is more evident in non-pregnant mares than in pregnant mares, suggesting that these lesions may influence the ability of mares to become pregnant (Tsutsumi et al. 1979). But lymphocytic infiltration in the oviduct is not always accompanied by uterine infiltration. Miragaya et al. (1997) found no significant correlation coefficients between the number of inflammatory cells in the oviduct and the number of inflammatory cells in the endometrium.

Given all the events that occur in the oviduct and the pathologies that it may suffer from, the objective of this work is to determine if the presence of pathologies has any relationship with the reproductive seasonality of mares.

2 | Materials and Methods

2.1 | Animals

The reproductive tracts of 59 mares that had been humanely slaughtered at an abattoir located at parallel 41° 39´´ north in northern Spain were used. Reproductive tracts were recovered within 10 min after they were removed from the mares and examined visually to discard those from pregnant mares. They were transported to the laboratory in a period of less than one hour at a controlled temperature of 22°C. Samples were collected throughout the year to obtain reproductive tracts in different reproductive seasons: January (anoestrus), April (spring transition/final spring transition), May (reproductive season) and the end of October (autumn transition). Since they were slaughterhouse animals and there was no access to their information, it was impossible to know their age or reproductive history and background.

To develop the experimental, favourable evaluation by the Ethics Advisory Committee of the University of Zaragoza (PD24/21NE) was requested, although the protocol is excluded from the scope of RD53/2013.

2.2 | Processing of the Reproductive Tracts

Separation of the ovary and its oviduct from the reproductive tract was performed by sectioning the apical zone of each uterine horn. Then, oviduct dissection was carried out to isolate it from the rest of the reproductive tract, and measurements were taken.

At the same time, the ipsilateral ovary activity of each oviduct was determined. For the ovarian activity evaluation, the presence of follicles (5–20 mm, 20–30 mm, >30 mm), corpus luteum and

Corpus albicans was recorded (Figure 3). The measurement of the different structures was made with a vertical calliper.

Afterward, the presence of oviductal pathologies was assessed, including adhesions, thickenings and cysts (ovarian or oviductal).

2.3 | Oviduct Flushing and Content Evaluation

The obtained oviducts, after being washed with PBS, were flushed from the infundibulum to the utero-tubal papilla with 10 mL of 5% methylene blue in saline solution to check for permeability, using a 10 mL syringe connected to a 14 g needle to canalise it (Figure 4). To collect the samples, the papilla was placed in a 15 mL Falcon tube, and the washing medium fell on it.

The recovered samples were centrifuged for 10 min at 10 x g, and 30μl of the pellet was extended and stained with Diff-Quik. The samples were spread on a slide and air-dried. Then, the samples were placed in each of the following solutions for 1 min: a fixative solution, an eosinophilic solution and a basophilic solution.

Once the samples were stained, they were evaluated using a microscope at 100X magnification.

Evaluation of cellularity was performed to observe the types of cells present in three fields, each representing one-third of the samples. The classification of cell types is shown in Table 1.

2.4 | Statistical Analysis

The statistical analysis was carried out using the IBM SPSS statistical package version 22.0. Contingency tables were calculated and analysed using the likelihood ratio test. For the rest of the variables, the Kruskal- Wallis test was performed and the Mann–Whitney test was applied to paired data where significant differences ($p < 0.05$) between the means were evident.

Finally, Spearman's correlation coefficient (rho coefficient) was calculated to establish the correlations between ovarian activity and the presence of different types of cells in the oviduct.

3 | Results and Discussion

3.1 | Ovarian Activity

The ovarian activity found throughout the different reproductive seasons during the year is as expected (Table 2).

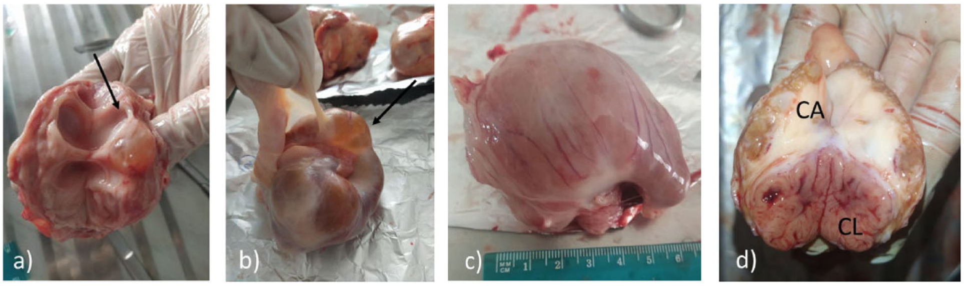

FIGURE 3 | (a) follicles 5 to 20 mm, (b) follicles 20 to 30 mm, (c) follicles >30 mm and (d) corpus luteum (CL) and corpus albicans (CA).

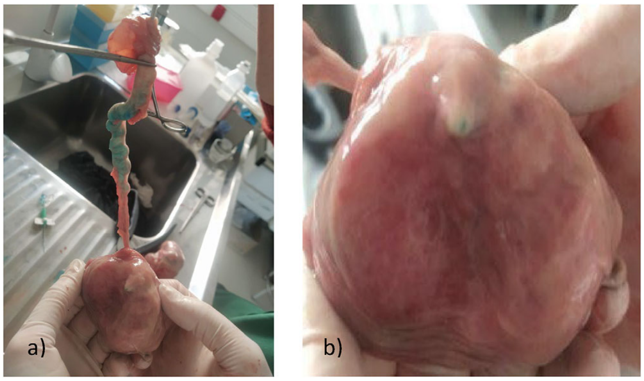

FIGURE 4 | (a) Oviduct flushing with 5% methylene blue and (b) An utero-tubal junction that is stained blue.

TABLE 1 | Cytological classification based on the number of different cell types.

	1	2	3	4
Cell number	0–50	50–100	100–200	>200
Ciliated cells	0–20	20–60	60–150	>150
Squamous cells	1–10	10–20	20–40	>40
Inflammatory cells	1–10	10–20	20–40	>40
Red blood cells	1–50	50–150	150–300	>300

When comparing both the right and left ovaries to see if there were differences in ovarian activity according to the time of the year, significant differences ($p = 0.008$) were found only during anoestrus in relation to the presence of corpus albicans, with a greater number of corpus albicans being observed in the left ovary (1.69 ± 1.195) compared to the right ovary (0.63 ± 0.719). This may be because ovulations occur to a greater extent in the left ovary, as previously suggested in literature (Hughes et al. 1980).

3.2 | Seasonal Changes in Permeability

The permeability of the oviducts was confirmed by assessing the staining of the utero-tubal papilla and assessing the amount of lavage medium recovered (Table 3).

By analysing oviduct permeability according to the time of the year, we observed that there were significant differences at different seasons, with the oviducts being less permeable during the spring transitional period; this was also the period with the highest percentage of partially patent oviducts, although most oviducts showed complete patency, without partial patency. Oviductal block is considered the most likely cause of unexplained infertility in mares, possibly due to the presence of collagen masses (Inoue and Sekiguchi 2018). During anoestrus, 100% of the oviducts were permeable. At the end of the spring transitional period, the percentage of permeable oviducts was high too, and especially during the reproductive season (91.2%). During the autumn transitional period, permeability began to decrease again to 87.5%, a percentage that was similar to that at the end of the spring transitional period, but higher than that during the spring transition. The season with more oviducts with no permeability resulted in the end of the spring transitional period, possibly due to the oviduct's own secretions that were preparing it for the reproductive season. The presence of collagen masses is associated with recent ovulation when part of the protein-rich connective tissue and follicular fluid passes into the oviduct, where it is retained, and collagen synthesis is generated around it (Lantz et al. 1998). This coincides with the findings of our study, which showed that the highest permeability occurred in anoestrus when there was no ovarian activity and, therefore, ovulation did not occur.

3.3 | Seasonal Changes in Adhesions and Thickening

In mares, embryos remain in the oviduct longer than in other species, reaching the blastocyst stage there and then passing into the uterus (Freeman et al. 1991). For this reason, it is very important that the oviduct be in optimal conditions to promote embryonic development. So, in addition to permeability, the presence of different anomalies was determined.

The presence of ovarian and oviductal adhesions, and the thickening or fibrosis of the oviduct was assessed (Figure 5). When quantifying them based on the season, the pathologies found presented significant differences (Table 4).

Although the pathogenesis of adhesions that cross the oviduct is unknown, and their effect on fertility has not been reported, it is possible that they may physically constrict the oviduct and interfere with fertility. In our study, we observed that both ovarian and oviductal adhesions showed a significant variation ($p < 0.05$) depending on the time of the year in which the samples were collected (Table 4). The incidence of adhesions between the ovary and the oviduct was higher during anoestrus, the

TABLE 2 | Presence of different follicular structures, corpus luteum and corpus albicans during different seasons. Different letters within each column denote statistical differences ($p < 0.050$); data are presented as means ± SEM.

Season	N	Follicle 5–20 mm	Follicle 20–30 mm	Follicle > 30 mm	Corpus luteum	Corpus albicans
Anoestrus (January)	16	2.00 ± 3.011[b]	0.88 ± 1.147	0.44 ± 0.814	0.13 ± 0.342[a]	1.25 ± 1.125[b]
Spring transition (early April)	22	1.27 ± 1.609[b]	1.18 ± 2.085	0.64 ± 0.902	0.14 ± 0.468[a]	0.00 ± 0.000[a]
Final spring transition (end of April)	14	4.07 ± 3.316[a]	2.43 ± 2.311	0.64 ± 0.497	0.43 ± 0.852[ab]	0.43 ± 0.646[a]
Reproductive season (May)	34	1.09 ± 1.712[b]	1.09 ± 1.379	0.76 ± 1.046	0.56 ± 0.705[b]	0.29 ± 0.462[a]
Autumn transition (end of October)	32	0.81 ± 1.120[b]	1.06 ± 1.014	0.5 ± 0.672	0.22 ± 0.42[ab]	1.16 ± 1.110[b]
	p-value	0.001	0.104	0.55	0.023	0.001

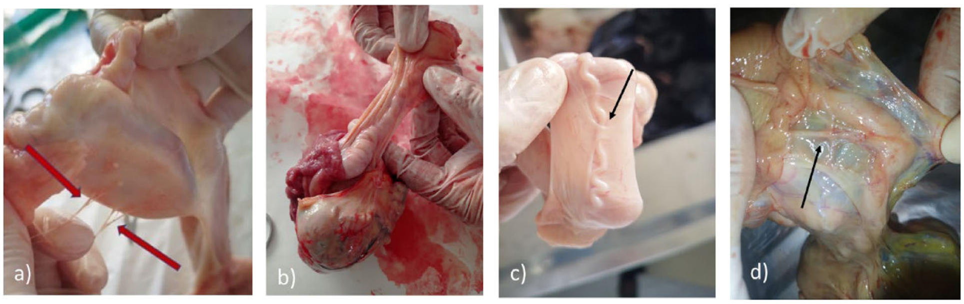

FIGURE 5 | Pathologies: (a) ovarian adhesions, (b) oviductal thickening and (c) and (d) oviductal adhesions.

TABLE 3 | Oviductal permeability across different seasons. Different letters in the columns indicate significant differences ($p < 0.05$).

Reproductive season	n	No	partial	complete
Anoestrus (January)	16	0.0%[b]	0.0%[c]	100%[a]
Spring transition (early April)	22	9.1%[b]	31.8%[a]	59.1%[b]
Final spring transition (end of April)	14	14.3%[a]	0.0%[c]	85.7%[a]
Reproductive season (May)	34	5.9%[b]	2.9%[b]	91.2%[a]
Autumn transition (end of October)	32	12.5%[a]	0.0%[c]	87.5%[a]
p-value			0.001	

spring transition, and at the end of the spring transition, while in the other seasons, ovarian adhesions were not found. Regarding oviductal adhesions, the highest percentage also occurred during anoestrus, where 18.8% of the mares' reproductive tracts showed adhesions, and during the spring transitional period. However, when entering the reproductive season, the incidence of oviductal adhesions decreased to 5.9%, and in the autumn transitional period, they completely disappeared, the same as ovarian adhesions.

Given that the main cause of adhesions in the reproductive tract is the presence of inflammatory or infectious processes in the abdominal cavity (Medenbachl et al. 1999), these values may be related to the incidence of digestive tract pathologies during the marked periods. It has been observed that the periods with the highest incidence are those in which food is poorer due to weather conditions; thus, adhesions may be related to the supplementation of concentrated food. Grain overload increases the risk of colic. High-grain diets decrease the water content of food intake in the colon due to decreased fibre content. Grains in the diet also increase gas production, altering motility and producing intestinal displacements (Gonçalves et al. 2002).

Some studies reported that oviductal adhesions are present in approximately 50% of the reproductive tracts recovered from slaughterhouse mares, but these studies did not specify the time of the year (Vandeplassche and Henry 1977, Saltie et al. 1986). This is a slightly higher percentage than ours if we look only at the percentage of adhesions present in the oviduct, which is 38.3% in our study. Oviductal adhesions are commonly associated with the infundibulum, but they can also be found transoviductal. In this case, if we incorporate the adhesions between the oviduct and the ovary together with oviductal adhesions, the percentage would be much higher in our case, at 78.5%. According to Stangroom and Weevers (1962), most adhesions are thin strips that are probably related to ovulation-associated blood loss, but in our study, adherences in the reproductive season are not present. At the moment it is not clear whether adhesions have a negative effect on oocyte uptake, transport and fertilisation. In a study by Saltiel et al. (1986), 72% of the mares that had adhesions were pregnant, in contrast 69% of the evaluated mares that showed failures in conception in the study of Pye et al. (2018) had perpendicular adhesions. These adhesions were presented unilaterally in 90% of the cases, and the oviducts were dilated in the area proximal to the constriction.

TABLE 4 | Ovarian and oviductal adhesions and oviductal fibrosis across different seasons. Different letters in the columns indicate significant differences ($p < 0.05$).

Reproductive season	n	Ovarian adhesions	Oviductal adhesion	Oviductal thickening
Anoestrus (January)	16	12.3%[a]	18.8%[a]	0%[b]
Spring transition (end of April)	22	13.6%[a]	13.6%[a]	0%[b]
Final spring transition (last April)	14	14.3%[a]	0%[c]	0%[b]
Reproductive season (May)	34	0%[b]	5.9%[b]	14.7%[a]
Autumn transition (end of October)	32	0%[b]	0%[c]	0%[b]
p-value		0.017	0.035	0.011

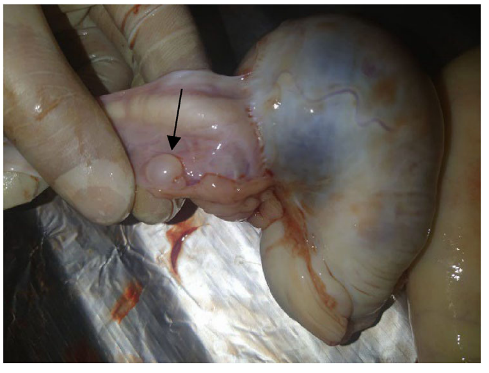

FIGURE 6 | Oviductal cyst.

Regarding the presence of adhesions in the left and right ovaries, we were able to confirm that there were no significant differences between them, except during anoestrus ($p < 0.028$) when the right ovary did not present adhesions of any kind, while the ovary on the left side did (37.5%), which could be justified by the thin activity of this ovary, as already demonstrated in previous research.

In relation to oviductal thickening, the only time during which thickened oviducts were found was the reproductive season, with an incidence of 14.7% (Table 3). In most cases, thickening is accompanied by inflammation, but this usually does not occur in mares (Bennett et al. 2002). In our study, oviductal thickening did not always occur bilaterally; in fact, thickening predominated in the left oviducts, with 29.40% of the left oviducts presenting thickening during the reproductive season ($p < 0.005$), thus coinciding with the greater activity of the left ovary. Other authors have also confirmed the presence of thickening in slaughterhouse pieces and verified that oviductal thickening is more common to appear as female mares' age (Saltie et al. 1986). This is an important fact to take into account because, according to Pinto et al. (2018) there seems to be a relationship between oviductal fibrosis and endometrial fibrosis, so in addition to endometrial pathology, fertility would also be compromised by oviductal pathology, which is always more difficult to diagnose.

3.4 | Seasonal Changes in Cyst Presence

The number of oviductal cysts (Figure 6) also showed significant differences depending on the reproductive season ($p < 0.05$).

TABLE 5 | Oviductal and paraovarian cysts across different seasons. Different letters in the columns indicate significant differences ($p < 0.05$).

Reproductive season	n	Oviductal cyst	Paraovarian cyst
Anoestrus (January)	16	0.00 ± 2.300[ab]	0.31 ± 0.602
Spring transition (early April)	22	0.41 ± 0.666[ab]	0.82 ± 0.958
Final spring transition (end of April)	14	0.29 ± 0.611[ab]	0.93 ± 1.269
Reproductive season (May)	34	0.29 ± 0.611[ab]	0.68 ± 1.319
Autumn transition (end of October)	32	0.72 ± 0.991[b]	1.16 ± 1.221
p-value		0.006	0.104

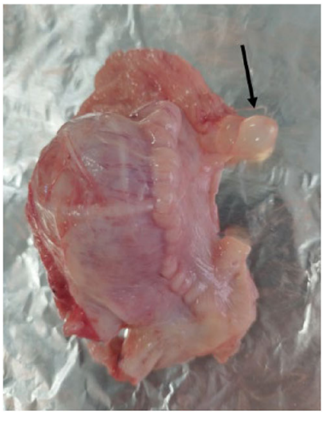

FIGURE 7 | Paraovarian cyst.

During the spring transitional period, more cysts of this type were found than in some other seasons, but the differences were not statistically significant. The autumn transitional period was the period with a significantly higher number of oviductal cysts. During anoestrus, no oviductal cysts were found (Table 5).

The presence of paraovarian cysts (Figure 7) had no significant differences between seasons, usually their presence is not relevant because they do not have endocrine activity, so no impact on fertility is present because they do not alter ovulation.

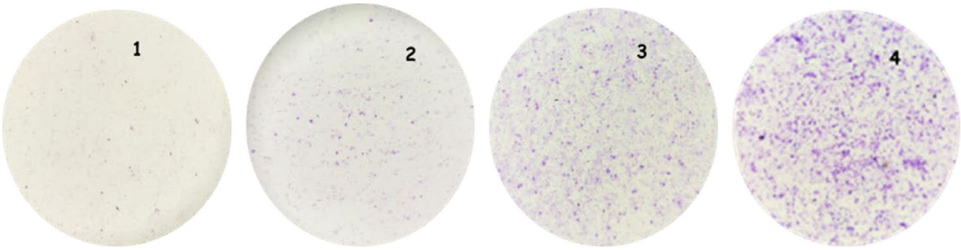

FIGURE 8 | Cellularity degree: 1 (0–50 cells), 2 (50–100 cells), 3 (100–200 cells) and 4 (>200 cells).

TABLE 6 | Differences depending on the season in the number of cells and the presence of ciliated, secretory, inflammatory and red blood cells found in oviductal fluid smears. Different letters in the columns indicate significant differences ($p < 0.05$).

Season	n	Cellularity	Ciliated cells	Secretory cells	Inflammatory cells	Red blood cells
Anoestrus (January)	16	2.31 ± 0.79^{a}	2.00 ± 0.73	1.00 ± 0^{a}	1.25 ± 0.45^{a}	2.31 ± 0.87^{ab}
Final spring transition (end of April)	14	2.09 ± 0.70^{a}	2.18 ± 0.60	1.00 ± 0^{a}	1.09 ± 0.30^{a}	2.18 ± 1.25^{ab}
Reproductive season (May)	34	3.06 ± 0.98^{b}	2.53 ± 0.84	1.03 ± 0.18^{a}	2.03 ± 1.03^{b}	2.78 ± 1.16^{b}
Autumn transition (end of October)	32	2.38 ± 0.90^{a}	2.04 ± 0.66	1.46 ± 0.71^{b}	1.08 ± 0.27^{a}	1.77 ± 0.99^{a}
p-value		0.003	0.050	0.001	0.001	0.009

They may be remnants of the Müllerian ducts from the time of embryological differentiation.

We might think that these cysts will cover the lumen of the oviduct, but oviductal occlusions are extremely rare in mares. However, there are studies that reported this situation of occlusion. Blue in 1985[30] carried out the collection and examination of embryos in the oviducts ipsilateral to the ovulated ovary in mares with the presence of these cysts. Thus, it can be assumed that the presence of these cysts could affect some of the processes that take place in the oviduct and, therefore, fertility. On the other hand, it has been observed that the presence of paraovarian cysts large enough to displace the fimbriae from an adequate position with respect to the ovary can affect fertility, since they can cause ectopic ovulation in the abdominal cavity (Bennett et al. 2002).

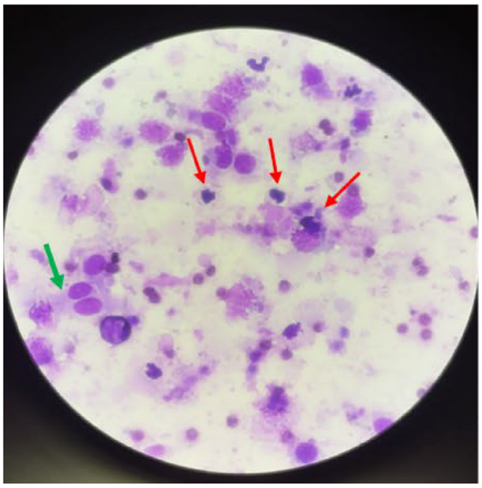

FIGURE 9 | Polymorphonuclear (red) and ciliated cells (green) with protein transudate during reproductive season.

3.5 | Oviductal Cytology

In our study, the criteria for the evaluation of cytology were the number of cells and the presence of inflammatory, ciliated, squamous epithelial and red blood cells (Table 1). These criteria were rated on a scale of 1 to 4 based on the approximate amount observed (Figure 8).

Regarding the variety of cells found, depending on the time of the year, we observed significant differences ($p < 0.05$) in the number of cells and the presence of squamous cells, inflammatory cells and red blood cells (Table 6).

Possible correlations between follicular activity and the type of cells obtained in the wash were analysed. In relation to follicular activity and cell type, we observed that there was a positive correlation between cellularity and the presence of corpus luteum (Rho = 0.236, $p = 0.030$) during the reproductive season. This situation might be due to the fact that, during this time, the genital system is more active and the rate of epithelial renewal is higher, in addition to the fact that the number of cells derived from ovulation, such as red blood cells, is greater than at other times. In a study conducted by Campbell et al. (1979) in which oviductal fluid was analysed during the reproductive season (April–August), the highest secretion rates were shown during the first seven days of the cycle, while the lowest secretion rates occurred during the middle of the cycle. Thus, the maximum secretion from the oviduct occurred before ovulation and decreased around the time of ovulation.

In our work, the presence of ciliated epithelial cells (Figures 9, 10, 11), desquamated epithelial cells (Figures 12, 13), lymphocytes (Figure 14) and occasional neutrophils (Figure 15) was found

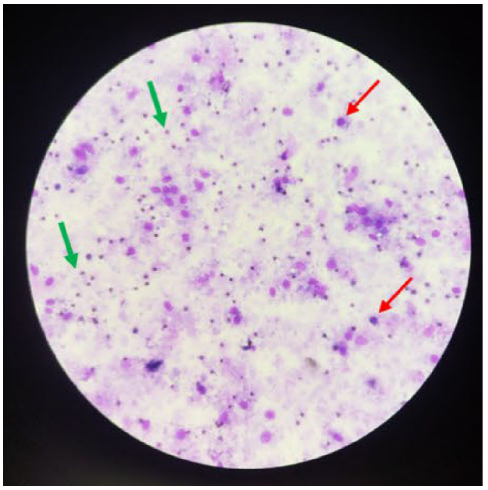

FIGURE 10 | Lymphocytes (red) and red blood cells (green) with protein transudate during reproductive season.

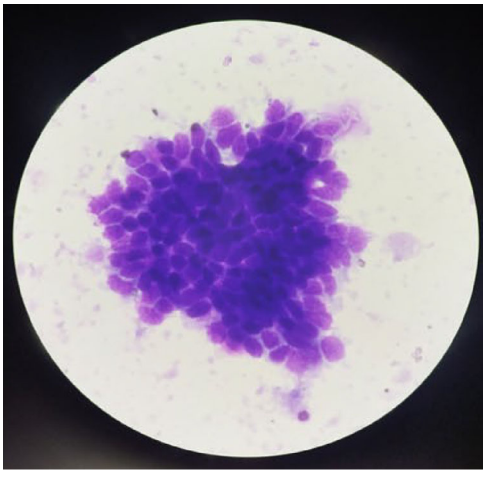

FIGURE 11 | Cell accumulation during the fall transitional season.

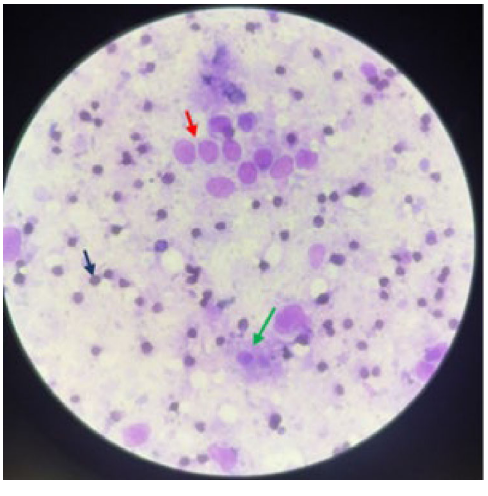

FIGURE 12 | Ciliated cells (red), lymphocytes (green) and red blood cells (blue) during reproductive season.

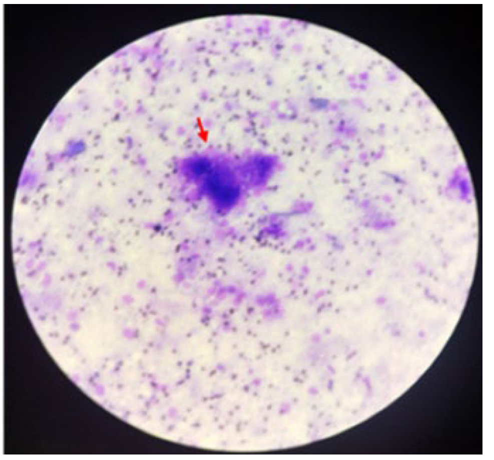

FIGURE 13 | Cell agglomeration during the fall transitional season.

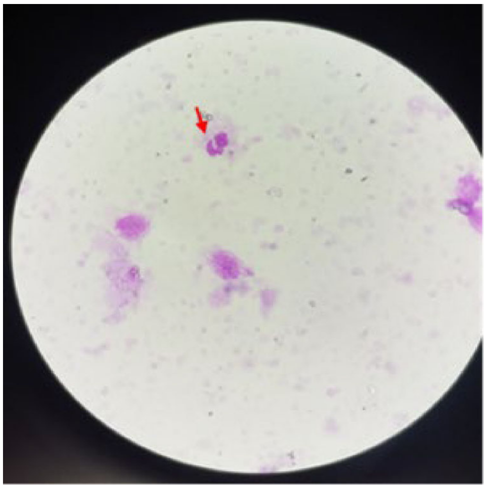

FIGURE 14 | Neutrophils during the fall transitional season.

to be common; in fact, there were no significant differences with regard to ciliated cells throughout the year. The number of squamous cells found in the smears was higher during the autumn transitional season, which is probably related to the regeneration of the epithelium after the summer reproductive activity.

The presence of inflammatory cells was significantly higher during the reproductive season. In fact, it was observed that at the end of the spring transition, there was a positive correlation (Rho = 0.638, $p = 0.638$) between the presence of inflammatory cells and the presence of corpora lutea. This might be due to the reaction against foreign bodies, such as the presence of sperm. In fact, in some of the extensions, the presence of spermatozoa could be observed. Even though the oviduct acts as a safe storage site for sperm (Fiala et al. 2007), and it has a separate environment from the uterus due to the presence of the utero-tubal papilla (Losinno et al. 1997), sperm is considered the main cause of physiological inflammation that occurs in the uterus after service (Kotilainen et al. 1964). During insemination, in addition to spermatozoa, foreign bodies, bacteria and debris enter the uterus directly, causing an inflammatory reaction known as post-service endometritis (Troedsson et al. 2001). Troedsson et al.

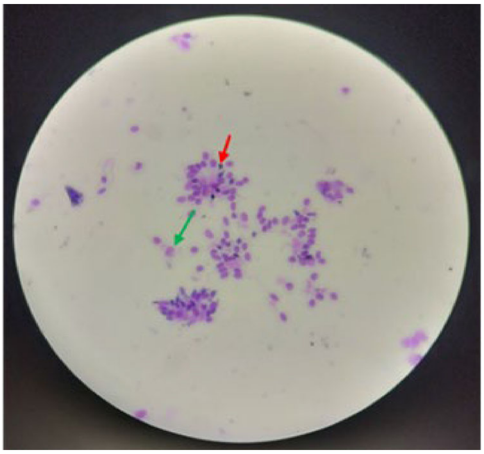

FIGURE 15 | Ciliated cells (green) and secretory cells (red) during the spring transitional season.

(1995) demonstrated that the sperm of equine species activates the complement, which results in the secretion of fluids with substances with chemoattractant properties, thereby initiating a rapid process of chemotaxis that attracts inflammatory cells (Pycock and Allen 1990) and leading to the subsequent massive invasion of polymorphonuclear cells from the blood into the uterine lumen. Cytokines are the most important inflammatory mediators, fulfilling functions such as recruitment of polymorphonuclear cells and activation of defence cells (Katila 2012). The area that is most frequently affected by inflammation is the region of the oviduct that is furthest from the uterus (Vandeplassche and Henry 1977). In our case, we make no differences between the different portions of the oviduct.

However, Fiala et al. did not find significant ($p > 0.05$) correlation coefficients between the number of inflammatory cells in the oviduct and the number in the endometrium in cyclic and noncyclic mares (Fiala et al. 2015).

The chlamydia presence could be another reason for inflammation. It has been observed that mares that have eosinophilic infiltration associated with salpingitis test positive for chlamydial antigen staining (Fiala et al. 2015). In fact, it is believed that chlamydia-induced infertility can lead to immune-mediated inflammatory processes in the oviduct. This, together with the epithelial degeneration caused by the chlamydial infection itself, may be what causes early embryonic death (Medenbachl et al. 1999).

When we assessed the presence of red blood cells (Figures 10, 14), we could observe that it was significantly higher during the reproductive season (Table 5). When correlations were established between the time of the year, ovarian activity and type of cells, significant correlations were only observed during anoestrus and the autumn transition in relation to the presence of red blood cells, with a positive correlation of 0.499 and a significance of $p = 0.049$ during anoestrus and a positive correlation of 0.439 during the autumn transition ($p = 0.025$). During the autumn transitional period, a negative correlation (-0.447) was observed between the presence of red blood cells and follicles >30 mm ($p = 0.022$). Finally, there was a positive correlation ($p = 0.301$) between the number of red blood cells and the presence of corpora lutea ($p = 0.005$).

This may be due to the presence of debris derived from the ovulation process since a large number of white blood cells and erythrocytes are released during ovulation and are propelled toward the lumen of the oviduct. In any case, the presence of an excessive number of erythrocytes may be due to debris formed as a result of sperm capacitation and the beginning of fertilisation. It is also possible that the vascularisation and permeability of the blood vessels that irrigate the genital apparatus during the reproductive season are increased to promote the secretion of substances belonging to the oviductal fluid or to facilitate the passage of hormonal substances to the genital apparatus since the production of secretions from the oviduct is under hormonal influence (Campbell et al. 1979); therefore, red blood cells can pass into the lumen of the oviduct via extravasation. The tissues of the female reproductive tract undergo cyclical physiological angiogenesis during the processes of ovarian folliculogenesis, corpus luteum formation and endometrial development. This physiological angiogenesis in the reproductive tract is regulated by proangiogenic and antiangiogenic factors (Costello et al. 2005).

Based on the results of our study, we can conclude that there is no correlation between oviductal permeability and the time of the year. Moreover, oviductal permeability has no relationship with the presence of pathologies, but an increased incidence of oviductal adhesions is observed during anoestrus and the spring transitional period. Regarding oviductal cellularity, it correlates with the ovarian activity observed throughout the year.

Author Contributions

Conceptualisation: Noelia González and Lydia Gil. *Methodology*: Noelia González, Agatha Varela and Lydia Gil. *Software*: Nacho de Blas. *Validation*: Noelia González, Agatha Varela and Lydia Gil. *Formal analysis*: Nacho de Blas. *Investigation*: Noelia González, Agatha Varela and Lydia Gil. *Resources*: Lydia Gil. *Data curation*: Nacho de Blas. *Writing – original draft preparation*: Noelia González. *Writing – review and editing*: Noelia González and Lydia Gil. *Supervision*: Noelia González and Lydia Gil.

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Ethics Statement

Favourable evaluation was made by the Ethics Advisory Committee of the University of Zaragoza (PD24/21NE), although the protocol is excluded from the scope of RD53/2013.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request. The data are not publicly available due to privacy or ethical restrictions.

Peer Review

The peer review history for this article is available at https://doi.org/10.1002/vms3.70753.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting File 1: vms370753-sup-0001-SuppMat.docx **Supporting File 2**: vms370753-sup-0002-SuppMat.docx **Supporting File 3**: vms370753-sup-0003-SuppMat.docx