APMB - Atti della Accademia Peloritana dei Pericolanti
Classe di Scienze Medico Biologiche
Vol. 113(1) 2025

ISSN 1828-6550

DOI: 10.13129 / 1828-6550 / APMB.113.1.2025.SD1

Scholarly Dialog **SD1 (1-12)**

Crosstalk between neuroendocrinology and follicular dynamism: the mare experimental model for reproductive "aging" in women*

Esterina Fazio

Department of Veterinary Sciences, Veterinary Physiology Unit, University of Messina, Messina, Italy

*Opening Lecture of the Annual Session of the Bio-Medical Class of the Accademia Peloritana dei Pericolanti-1729

Abstract

The study of reproductive physiology in relation to advancing age is of great interest and involves many species, evaluating the mare as a potential model to study follicular and oocyte maturation in the woman. As in women, the mare has a comparable timing with the events associated with follicular and oocyte maturation, having a long follicular phase, a large follicle size, and the timing between human chorionic gonadotrophin (hCG) o equine chorionic gonadotrophin (eCG) administration and ovulation nearly superimposable. Follicular dynamism is characterised by three functional moments whose endocrine mechanisms dictate their physiological sequence of events: Recruitment, selection and dominance. Aging had caused profound functional alterations at the ovarian level, counaltered luteal phase, LH and progesterone, reduced follicular phase, compensated by increased FSH and E_2, increased cycle duration and inter-ovulatory intervals, intermittent ovulations, and increased pool of growing primordial follicles, resulting in accelerated depletion of ovarian reserve.

This significant sequence of functional and neuroendocrine effects are the expression of impending reproductive senescence or menopause, respectively, in the mare and the woman in whom the reproductive life turns out to be twice as long as the mare. Aging thus represents a dissociation between oocyte maturation and ovulation, causes a decline in the quality of oocytes.

Older mares had lower androgen pattern than younger, showing that age induces reduction in androgens' synthesis in physiologically cyclic Spanish Purebred mares. In humans' experimental animals' and mares'over 16 years, components of the somatotropic axis, such as growth hormone (GH) and insulin-like growth factor 1 (IGF-1) concentrations, decrease with advancing age. Advanced age leads to a predominance of sympathetic nervous activity and lower serotonergic and dopaminergic activity in non-pregnant mares. The pivotal interaction between serotonin (5-HT) and calcium shift in aging pregnant and cyclic mares was recorded, with lower 5-HT, total calcium and ionized calcium in the oldest mares. Aging appears to reduce the secretory tone of 5-HT, with a concurrent large shift in calcium metabolism in pregnant mares. In woman and in animal models, as the mare, estrogens are involved in iron (Fe) homeostasis, supporting the hypothesis of the existence of an "estrogen-iron axis", with advancing aging. The correlation between thyroid hormones (TH) and ovarian pool was recorded. Ovulation representing a controlled inflammatory process is also mediated by the intervention of glucorticoids, especially cortisol. The ovary is the target tissue for glucocorticoids in many species, including women and mares, but it does not synthesize cortisol de novo.The use of in vivo rather than in vitro models, with a positive impact on unique human, animal and environmental health, is a desirable application for future research in this field.

Keywords: aging; mare; experimental mode, folliculogenesis, oocyte maturation

Corresponding Author: Esterina Fazio - esterina.fazio@unime.it

The harmonious realization of reproductive activity in the female relies on the anatomical, histological, biochemical, and hormonal integrity of the ovarian, hypothalamic, pituitary, and uterine structures involved.

Therefore, an understanding of the regulatory, endocrine, autocrine and paracrine mechanisms that enable the alternating phases of follicular and luteal growth is an indispensable pre-requisite to knowledge of reproduction and in particular the cyclicality of the female. The study of folliculogenesis and oocyte maturation in relation to advancing age is of great interest and involves many species that serve as experimental models, ranging from the mouse to the monkey (1). Various animal models have advantages and disadvantages for studying specific mean topics of reproductive physiology, particularly to human reproduction.

The nonhuman primate may represent one of the most appropriate models for the woman because of evolutionary relatedness and similarities in reproductive aging (2). Nevertheless, studies in nonhuman primates can be limited by availability of animals, costs, management considerations, and ethical concerns and approaches. The majority of research on reproductive aging effect has included rodent models, which have advantages including ease of management, space considerations, homogeneity of population, rapid aging and relative ease of genetic manipulation. Moreover, compared to women, rodents are multiparous species, with a short life span, rapid progression of reproductive processes and differences in reproductive endocrine trends. The uniformity of the mouse models, while allowing for homogeneous sample collection, greatly limits their inter- and intra-individual variations, and making such a model limiting (3).

Many other animal species have been used as reproductive aging in women, from follicular and luteal phases to endocrine characteristics, like the bovine (4), or ovine species; however, their limitation is related to the fact that few such animals are kept alive but no longer productive, with significant economic repercussions. Primarily ethical, as well as economic, constraints limit the use of nonhuman primates.

In contrast to many domestic species, mares in which it is not uncommon to find a fairly large population of broodmares over 20 years of age, often used as working or companion animals, providing a population of mares in which oocytes have been maintained in meiotic arrest for decades (3). Another variable of absolute importance lies in the fact that many farms and stables are often located in urban and suburban areas, creating a completely overlapping environmental setting in the two comparatively studied species, equine and human. It is precisely the same environmental stresses, which will influence the molecular, genetic, population and thus community aspect, affecting both reproduction and fertility.

The mare is also anatomically different from the woman, but these differences are more of a strength rather than a limitation. The mare has a bicornuate uterus, due to incomplete fusion of Muller's ducts, compared to the woman who possesses a simple uterus, resulting from complete fusion of the aforementioned ducts. A woman's ovary has an irregular shape and is characterized by an outer portion, the cortical, featuring the germ line, and a central portion, the medullary, rich in connective tissue, vessels, and nerve endings. In contrast, the mare ovary has a regular shape, and is characterized by an internalized cortical, almost occupying medullary, and oriented toward the ovulatory dimple, a species peculiarity; it is at this level that ovulation takes place, since here the ovary is not covered by the albuginea tunica.

However, the uterine dimensions, together with the entire reproductive system, in the mare make it an optimal model for both easily assessable for palpation and ultrasound, with the potential for repeated observations and follicular manipulations. As in women, the mare has a long follicular phase, allowing studies of follicular growth, deviation, and regression, evaluating the mare as a potential model to study follicular and oocyte maturation and reproductive aging in the woman (3,5,6).

Follicular dynamism in the woman as in the mare is characterized by the frenetic succession of functional moments, under the regimented endocrine control of the hypothalamic-pituitary-ovarian axis. Follicular growth is characterized by the proliferation and differentiation of theca and granulosa cells, induced by hormonal stimuli, resulting in an increase in the capacity of follicles to produce estradiol and respond to gonadotropins. The factors responsible for the initiation of primordial follicle growth are not yet fully elucidated. Up to a certain stage, development seems to be independent of the presence of gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH). Gonadotropins, in fact, and especially the basal level of FSH, would seem to be more responsible for regulating the synthesis capacity and maturation of granulosa cells and not for follicle growth proper. Numerous studies show that follicular growth is the result of the interaction existing between the pituitary gonadotropic hormones FSH and LH and the polypeptide substances present in the follicle (inhibin, activin, follistatin, etc.) (7).

Follicular dynamism is characterised by three functional moments whose endocrine mechanisms dictate their physiological sequence of events. Recruitment is the initiation of terminal growth of a group of gonad-dependent follicles. A gonad-dependent follicle is one that has passed the stage where most follicles become atretic. It is a “random” mechanism whereby follicles that have reached “good size at the appropriate time” are recruited. Recruitment is caused by the transient increase in FSH that acts on such follicles, increasing their aptitude to aromatize androgens into estrogens. Selection is subsequent to the reduction of FSH that marks the initiation of recruitment. In fact, the development of a group of recruited follicles is associated with increased production of Estradiol (E_2) and inhibin, hormones, both of which exert negative retro-control on FSH production, which then decreases. When FSH concentration becomes lower than that which induced recruitment, the recruited follicles enter atresia, except for the selected follicle and the mechanism of ovulatory follicle selection is not yet known. Dominance follows selection. Dominance, therefore, corresponds to blocked recruitment and rapid volumetric growth of ovulatory follicles. Despite declining FSH, the follicle persists as it acquires an internal self-stimulation mechanism: E_2 produces amplifies Insulin growth factor 1 (IGF1) synthesis. IGF1, in turn, it stimulates the aromatization of androgens into estrogens. In addition, granulosa acquisition of receptors for LH, associated with active LH secretion, contributes to the maintenance of a high concentration of AMPc in follicular cells and thus to dominant follicle growth (7).

Hence, follicular growth is the result between the gonadotropic hormones FSH and LH, of pituitary origin, and the polypeptide substances present in the ovary: inhibin, activin, follistatin that exert negative feedback on the pituitary gland; but they can also exert a 'direct action on the ovary. Therefore, when FSH has reached the minimum level, the deviation of the dominant follicle occurs, which, in the mare, has a diameter of about 10 mm and in the woman of 10 mm; this represents the minimum size that a follicle must have to ensure a sufficient number of LH receptors in order to ovulate. Moreover, more specifically FSH receptors are present only on the granulosa cells, whereas LH receptors are also present on the theca cells of the antral follicle. In the dominant follicle, LH receptors are in greater numbers than those present on the subordinate follicle, and this explains why the dominant follicle, at the time of the minimum FSH level, deviates becoming the dominant follicle, while the other follicles go into regression (7).

The large size of equine follicles provides excellent intra-follicular experimental access for in vivo experimental manipulations, such as intra-follicular treatment, sequential sampling of follicular fluid (5), and monitoring of pre-deviant vascular changes by colour ultrasound Doppler (8). The present results also encourage comparative study between the two species on the similarities and differences in systemic and local physiological changes associated with follicle morphological dynamics.

The differences between mares and women in the dynamics of follicle development during the inter-ovulatory interval and during the ovulatory follicular wave include the following characteristics, on the basis of results recorded by Ginther et al., (6): a more complex ovulatory follicular wave in mares than in women, due to the greater number of follicles per wave and the greater heterogeneity of follicles from previous waves; higher growth rate of the dominant follicle after deviation than before deviation in women, but not in mares; higher rate of regression of non-dominant follicles in women.

Similarly, again from the studies of Ginther et al, (6) similarities emerge from the appearance between mare and woman, given below: the appearance of the future dominant follicle before the future larger subordinate follicle, similar time intervals between the sequential appearance of follicles, a similar rate of follicle growth during the common growth phase maintenance of the mare: woman ratio in the diameter of the dominant follicle (about twice as large in mares) from the onset of deviation to ovulation, similar incidence of pre-deviated follicles during ovulatory waves, similar incidence of major anavulatory waves during the inter-ovulatory interval.

Comparative studies conducted by Carnevale (3) in older (15-19 years old) and younger (3-7 years old) mares compared with adult (40-45 years old) and young (20-25 years old) mares yielded very interesting indications. Aging had caused profound functional alterations at the ovarian level, co-unaltered luteal phase, LH and progesterone, reduced follicular phase, compensated by increased FSH and E_2, increased cycle duration and inter-ovulatory intervals, intermittent ovulations, and increased pool of growing primordial follicles, resulting in accelerated depletion of ovarian reserve.

This significant sequence of functional effects and events are the expression of impending reproductive senescence or menopause, respectively, in the mare and the woman in whom the reproductive life turns out to be twice as long as the mare. Moreover, reproductive cycles cease at 25 years in the mare and at 50 years in the female (3). Likewise, in 20-24-year-old mares, it was described that 19% presented absence of ovulation and follicular growth, while 37% presented absence of ovarian activity which, in Ponies, increased to 50%. Likewise, in 46-year-old women, a 10-fold reduction in the number of primordial follicles was observed.

Why choose the mare as the ideal experimental model in folliculogenesis and follicular growth? Because the mare is a seasonal polyestral species, with a long photoperiod and spontaneous ovulation, in which longer daylight hours, associated with increased serotonin, secreted by the pineal, activates the hypothalamus-pituitary-ovary axis, initiating the breeding season. Therefore, the mare alone constitutes a threefold experimental model, being able to study it during its abnormal cyclicality, during the quiescent phase of the HPA-ovary axis (winter anaestrus), and during the transitional phase (initiation to the cyclic phase or onset of anaestrus).

Why choose the mare as the ideal experimental model in folliculogenesis and follicular growth? Because the mare has a comparable timing with the events associated with follicular and oocyte maturation, because it has a long follicular phase, because the follicles are large in size, and because the timing between human chorionic gonadotrophin (hCG) o equine chorionic gonadotrophin (eCG) administration and ovulation is nearly superimposable.

Aging thus represents a dissociation between oocyte maturation and ovulation, which in the 14-year-old mare compared to the 2-11-year-old mare has a conception rate of 31% vs 57%, a birth/season rate of 48% vs 82%, a 12-day pregnancy rate of 32% vs 100%, and an embryo loss rate of 62% vs 11%. Likewise, increased rates of chromosomal abnormalities have been described in older women, in whom more miscarriages occur, ranging from 13% in young women aged 35 years to 53% in women older than 42 years.

Aging also causes a decline in the quality of oocytes that have more vesicles inside them, the average number of vesicles degrees/oocyte also increases, morphological abnormalities appear, mitochondrial DNA mutations, uncontrolled production of reactive oxygen species (ROS).

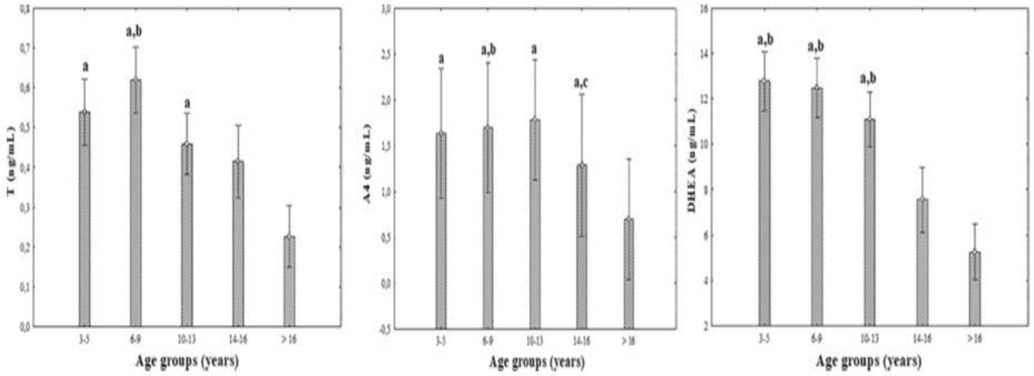
Recently, the total change undergone by the ovary as a result of aging has been described in detail (9), with decline in follicular reserve, the appearance of pro-inflammatory mediators and ROS, collagen deposition, mitochondrial dysfunction, and accumulation of multinucleated giant cells. Likewise, during gestation, there is a higher energy demand which increases oxygen requirements, with an elevated production of reactive oxygen species. Notably, unlike in equines, the effects of maternal age and parity on women's oxidative profiles are well-documented. Thus, the oxidative changes in mares during the last third of pregnancy and the postpartum period, considering age and parity were recently investigated (10). Antioxidant defenses were measured through glutathione peroxidase, glutathione reductase, and superoxide dismutase activities. Results indicated that glutathione peroxidase levels were higher in older mares, showing an aging effect; conversely, glutathione reductase, superoxide dismutase, lipid and protein oxidation, offering insights into the role of maternal age in antioxidant mechanisms.

Why choose the mare as the ideal experimental model in folliculogenesis and follicular growth?

Because the presence of aromatase in the follicular antrum has been described in the 6-month-old filly, such that mare ovaries are able to synthesize E_2 long before the first ovulation. Moreover, the expression of androgen receptors on ovarian cells (11).

Androgens are produced in both sexes. In females produced by the adrenal gland and the ovaries they play a crucial role in regulating ovarian function, synthesis of estrogens and follicular growth. Age leads to a reduction in androgen concentrations, although, at present, these mechanisms are not elucidated in mares. The evaluation of the concentrations of testosterone (T), androstenedione (A_4) and dehydroepiandrosterone (DHEA) in mares of different ages (3–5 years, 6–9 years, 10–13 years, 14–16 years and > 16 years) is reported in **Figure 1**.

Fig. 1 Mean ± SD of Testosterone (T), Adrostenedione (A_4) and Deidroepiandrosterone (DHEA) in mares of different age groups

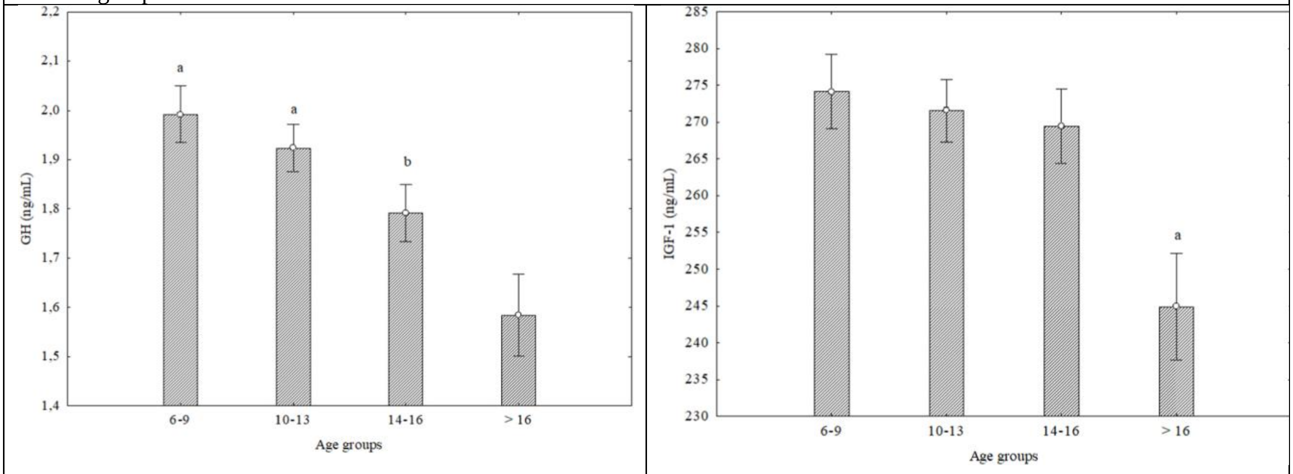


Letters indicate significant differences among age groups: a = vs > 16 years (P < 0.05); b = vs 14–16 years (P < 0.05); c = vs 10–13 years (P < 0.05 (By Satué et al., 2024a)

Older mares had lower androgen pattern than younger, showing that age induces reduction in androgens' synthesis in physiologically cyclic Spanish Purebred mares (12).

In humans' and experimental animals' components of the somatotropic axis, such as growth hormone (GH) and insulin-like growth factor 1 (IGF-1) concentrations, decrease with advancing age. Although there is evidence regarding IGF-1, the effect of age on GH in mares, as well as the relationships between both parameters, have not yet been elucidated. Hence, the objective of this study was to evaluate the concentrations of GH, IGF-1, E_2, and P_4 in mares, according to the different ages (6–9 years, 10–13 years, 14–16 years and >16 years) **Figure 2**.

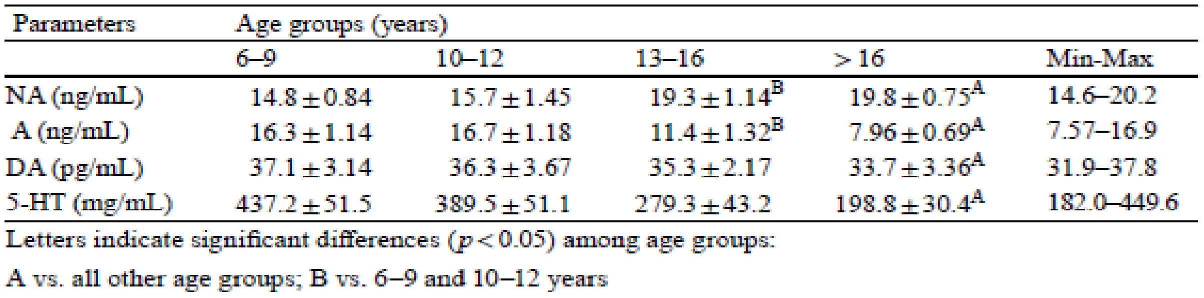
Fig. 2 Mean ± SD of Growth Hormone (GH) and Insulin Growth Factor (IGF-1) in mares of different age groups



Letters indicate significant differences among age groups: a: vs 14–16 and >16 years; P < 0.05 b: vs > 16 years; P < 0.05. (By Satué et al., 2024bb)

The concentrations of E_2 and P_4 did not change with age (**Table 1**).

Table 1 Mean ± SD of Noradrenaline (NA), Adrenaline (A), Dopamine (DA) and Serotonin (5-HT) in mares of different age groups



Parameters	Age groups (years)				
	6–9	10–12	13–16	> 16	Min-Max
NA (ng/mL)	14.8 ± 0.84	15.7 ± 1.45	19.3 ± 1.14^{B}	19.8 ± 0.75^{A}	14.6–20.2
A (ng/mL)	16.3 ± 1.14	16.7 ± 1.18	11.4 ± 1.32^{B}	7.96 ± 0.69^{A}	7.57–16.9
DA (pg/mL)	37.1 ± 3.14	36.3 ± 3.67	35.3 ± 2.17	33.7 ± 3.36^{A}	31.9–37.8
5-HT (mg/mL)	437.2 ± 51.5	389.5 ± 51.1	279.3 ± 43.2	198.8 ± 30.4^{A}	182.0–449.6

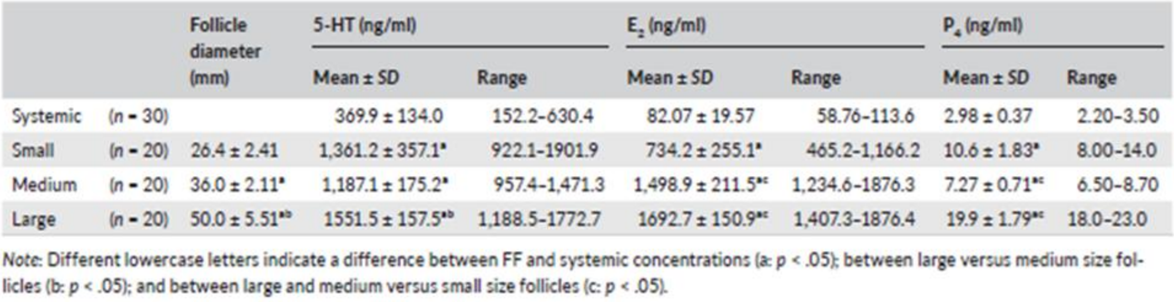
Letters indicate significant differences ($p < 0.05$) among age groups:
A vs. all other age groups; B vs. 6–9 and 10–12 years

(By Satué et al., 2024b

Advancing age leads to a decrease in the activity of the somatotropic axis in physiological cyclic mares, represented by a significant GH reduction, which, however, was ascribed for IGF-1 exclusively to mares over 16 years of age, without alterations in steroid hormone patterns (13).

Special attention was given to the aging effect on the adrenergic, serotonergic, and dopaminergic systems in the mare. Since the expression of beta adrenergic receptors has been described in both theca and granulosa, it seemed interesting to evaluate catecholamine changes with aging in the mare. Catecholamines (CATs) are neurotransmitters and allostatic hormones whose plasma concentrations are physiologically modified in various species such as human, rats, mice and donkeys, with advancing age. However, currently these mechanisms are less well elucidated in horses and more specifically in mares. The hypothesis of this study was that, as in afore mentioned species, the CATs could experience physiological changes with advancing age. The objective of this study was to evaluate the concentrations of adrenaline (A), noradrenaline (NA), dopamine (DA), and serotonin (5-HT) in mares of different ages (6 to 9 years, 10 to 12 years, 13 to 16 years and > 16 years). Advanced age leads to a predominance of sympathetic nervous activity and lower serotonergic and dopaminergic activity in non-pregnant mares (14) (**Table 2**).

Table 2 Mean ± SD of follicle diameter and systemic and intrafollicular serotonin (5-HT), oestradiol-17ß (E2) and progesterone (P4) concentrations regarding different categories of follicle size in mares



		Follicle diameter (mm)	5-HT (ng/ml)		E_2 (ng/ml)		P_4 (ng/ml)	
			Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Systemic	(*n* = 30)		369.9 ± 134.0	152.2-630.4	82.07 ± 19.57	58.76-113.6	2.98 ± 0.37	2.20-3.50
Small	(*n* = 20)	26.4 ± 2.41	1,361.2 ± 357.1^{a}	922.1-1901.9	734.2 ± 255.1^{a}	465.2-1,166.2	10.6 ± 1.83^{a}	8.00-14.0
Medium	(*n* = 20)	36.0 ± 2.11^{a}	1,187.1 ± 175.2^{a}	957.4-1,471.3	1,498.9 ± 211.5^{ac}	1,234.6-1876.3	7.27 ± 0.71^{ac}	6.50-8.70
Large	(*n* = 20)	50.0 ± 5.51^{ab}	1551.5 ± 157.5^{ab}	1,188.5-1772.7	1692.7 ± 150.9^{ac}	1,407.3-1876.4	19.9 ± 1.79^{ac}	18.0-23.0

Note: Different lowercase letters indicate a difference between FF and systemic concentrations (a: $p < .05$); between large versus medium size follicles (b: $p < .05$); and between large and medium versus small size follicles (c: $p < .05$).

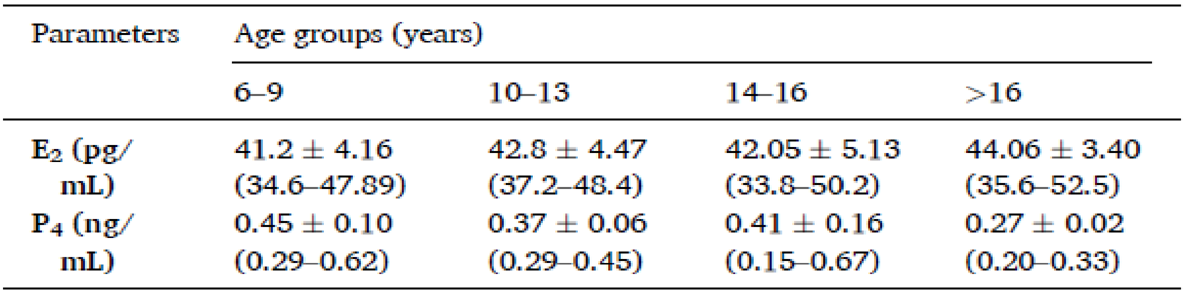
(By Satué et al., 2019)

It is known, however, that DA and NA exert a role in early follicular recruitment but not in late antral follicle. In contrast, A is present in follicular development, up to the pre-ovulatory stage. DA is present, however, in higher concentrations in the medium-sized follicle than in the large follicle. Moreover, the ovary expresses type 2 dopaminergic receptors at the level of ovarian tissue, but not antral follicle, and type 1 at the level of corpus luteum. DA increases significantly in the cerebrospinal fluid of mares in anaestrus compared with the breeding season. In addition, administration of a DA antagonist induces cyclicality initiation in mares in anaestrus or in the transition phase.

Granulosa cells form a functional barrier to 5-HT in the growing ovarian follicle. Circulating 5-HT in maternal blood is the main source of 5-HT in the ovary. At the same time, the growing oocyte is able to accumulate 5-HT with the help of the membrane transporter SERT. Between these two points is a layer of granulosa cells that take up 5-HT and degrade it with the help of monoamine-oxidase (MAO). This creates a functional barrier for 5-HT and isolates growing oocytes from it until ovulation (15).

Moreover, a bidirectional crosstalk between ovarian steroids and the follicle serotonergic system has recently been demonstrated. Serotonin promotes gonadotropin releasing hormone (GnRH) secretion, promotes follicular growth and oocyte maturation, regulates antral follicle growth and development, and promotes steroidogenesis, angiogenesis, and corpus luteum development. The hypothesis that a local serotonergic network might also exist in the follicle of mares remains poorly documented, with exception for humans and laboratory species. The intrafollicular serotonin concentrations of the cycling mare at ovulation time was recorded. Sixty ovaries collected from 30 clinically healthy mares of slaughterhouse meat production with clinically normal reproductive tracts after slaughtering were evaluated. Blood samples were taken prior to sacrifice. Follicles were classified in three categories in relation to size, as small (20–30 mm), medium (31–40 mm) and large (>41 mm), and the follicular fluid samples were extracted from each follicle. Intrafollicular 5-HT, E_2 and P_4 concentrations were higher than systemic. 5-HT concentrations increased in largest compared to medium follicles, without differences compared to small size follicles (**Table 3**).

Table 3 Mean ± SD of oestradiol-17ß (E2) and progesterone (P4) concentrations in the different age groups



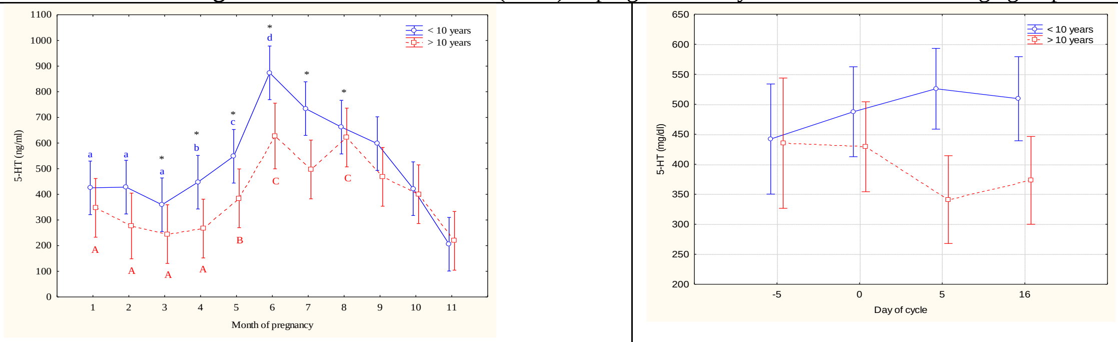
Parameters	Age groups (years)			
	6–9	10–13	14–16	>16
E_2 (pg/ mL)	41.2 ± 4.16 (34.6–47.89)	42.8 ± 4.47 (37.2–48.4)	42.05 ± 5.13 (33.8–50.2)	44.06 ± 3.40 (35.6–52.5)
P_4 (ng/ mL)	0.45 ± 0.10 (0.29–0.62)	0.37 ± 0.06 (0.29–0.45)	0.41 ± 0.16 (0.15–0.67)	0.27 ± 0.02 (0.20–0.33)

(By Satué et al., 2023a

5-HT and E_2 and 5-HT and P_4 were positively correlated. 5-HT and P_4 concentrations in follicular fluid increased progressively with the increase in follicular size. Follicle diameter and E_2 and P_4 were correlated. Since 5-HT interacts with steroids, its role on steroidogenesis during growth of the dominant follicle may be suggested (16).

Moreover, the pivotal interaction between 5-HT and calcium shift in aging pregnant and cyclic mares was recorded, with lower 5-HT, total calcium and ionized calcium in the oldest mares. Aging appears to reduce the secretory tone of 5-HT, with a concurrent large shift in calcium metabolism in pregnant mares (17) (**Figure 3**)

Fig. 3 Mean ± SD of Serotonin (5-HT) in pregnant and cyclic mares of different age groups

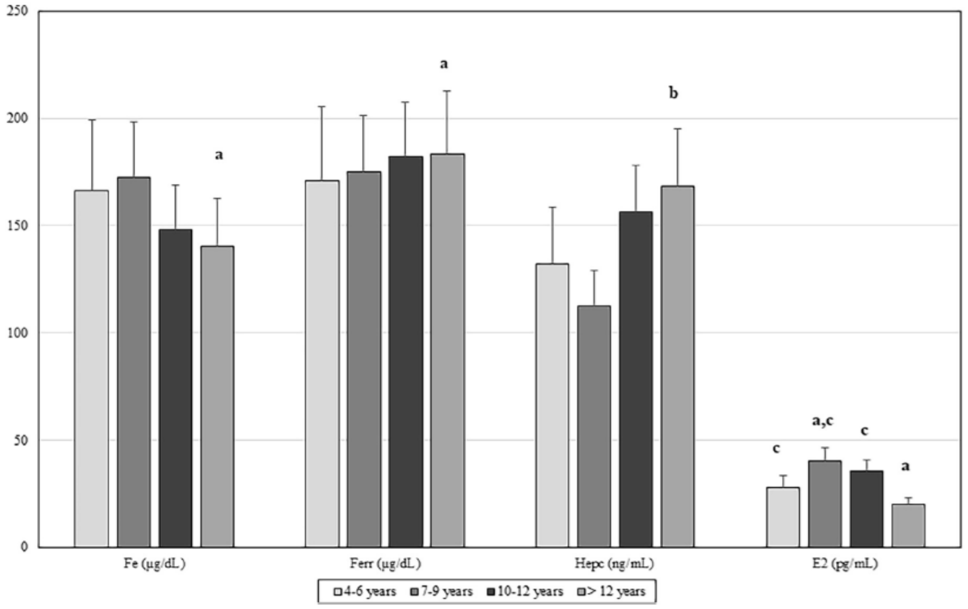


Symbol indicates significant differences *vs* >10 years: mares aged <10 showed higher 5-HT concentration from 3rd to 8th month of pregnancy ($P < .05$)

(By Satué et al., 2025, in press)

The maintenance of iron (Fe) homeostasis is vital for the physiological function along life. In sexually mature humans and experimental animals, estrogens downregulate hepcidin (Hpc) expression, in order to improve the intestinal absorption and to mobilize Fe stores for maternal erythropoietic expansion and placental development. However, changes of these mechanisms related to regulation of Hpc on the availability of Fe during gestation with advancing age in mares, remain unknown. In woman and in animal models, estrogens are involved in iron (Fe) homeostasis supporting the hypothesis of the existence of an “estrogen-iron axis”. Since advancing age leads to a decrease in estrogen concentrations, the mechanisms of Fe regulation could be compromised. In cyclic and pregnant mares, to date, there is evidence linking the iron state with estrogens pattern. Then, the relationship among Fe, ferritin (Ferr), Hepc and E_2 in cyclic mares (18) and pregnant mares with advancing aging was recorded (19) (**Figure 4**).

Fig. 4 Mean ± SD of Iron (Fe), Ferritine (Ferr), Hepcidina (Hepc) and oestradiol-17ß (E_2) in mares of different age



Letters indicatesignificant differences *vs* 4-6 years: a=P<0.01; *vs* 7-9 years: b= P<0.01; *vs* >12 years: c= P<0.01

(By Satué et al., 2023b)

There is a direct relationship between E_2 and Fe metabolism, mediated by the inhibition of Hepc in Spanish Purebred mares. The reduction of E_2 decreases the inhibitory effects on Hepc, increasing the levels of stored Fe and mobilizing less the free Fe in circulation. Based on the fact that ovarian estrogens participate in changes in the parameters indicative of iron status with age, the existence of an “estrogen-iron axis” in the mares'estrous cycle could be considered.

Equally important is the implication of thyroid hormones on reproductive activity. A correlation between serum T_4 and FF has been described in the mare, just as a correlation exists between 5'monodesiodase (5'MD) and T_3 in the FF; 5'MD in the FF is able to degenerate T_3 (paracrine effect) more in the ovulatory phase mare (20). In women, increased ovarian iodine uptake has been described during follicular growth, with a reduction instead in puberty and post-menopause or in the hypothyroid subject. The expression of Na-Iodine Symporter (NIS) at ovarian level was described and the presence of 5’MD makes the ovaries independent of thyroid changes. The correlation between THs and ovarian pool was recorded (11). THs also act on redox systems and oxidative stress, acquiring a predictive role on fertilization success, and with cytokines (IL1, IL6, INF and TNF) in the FF, hypothesizing their involvement in peri-ovulatory inflammatory process and oocyte quality.

Ovulation representing a controlled inflammatory process is also mediated by the intervention of glucorticoids, especially cortisol, with its direct non-genomic and indirect genomic anti-inflammatory actions. The ovary is the target tissue for glucocorticoids in many species, including women and mares, but it does not synthesize cortisol de novo. In women elevated cortisolemia in the FF, in coincidence of peak LH and 11 ß -hydroxy dehydrogenase 1 (11ß -HSD1), demonstrates its role in mediating apoptosis in the granulosa cells. In the mare, cortisol influences the expression of 11 ß-HSD2 in oophorus cumulus cells, promoting local glucocorticoid metabolism in the mare (21). Cortisol also correlates positively with P_4.

The choice of an ideal experimental model such as the mare for the processes of female folliculogenesis and oogenesis allows the use of in vivo rather than in vitro models, with a positive impact on unique human, animal and environmental health.

Conflicts of Interest. The Author declares no conflict of interest

References

1.vom Saal, F.S., Finch, C.E., Nelson, J.F. (1994). Natural history and mechanisms of reproductive aging in humans, laboratory rodents and selected vertebrates. In: Knobil E, Neill, et al., editors. The physiology of reproduction, vol. 2. New York: Raven Press, Ltd., pp. 1213–314.

2. Bellino, F.L., Wise, P.M. (2008). Nonhuman primate models of menopause workshop. Biol Reprod, 68 (1), 10–18. doi: 10.1095/biolreprod.102.005215

3. Carnevale, E.M. (2008). The mare model for follicular maturation and reproductive aging in the woman. Theriogenology, 69 (1), 23-30. doi: 10.1016/j.theriogenology.2007.09.011

4. Malhi, P.S. Adams, G.P., Singh, J. (2005). Bovine model for the study of reproductive aging in women: follicular, luteal, and endocrine characteristics. Biol Reprod, 73 (1), 45-53. doi: 10.1095/biolreprod.104.038745

5. Ginther, O.J., Gastal, E.L., Gastal, M.O., Checura, C.M., Beg, M.A. (2003). Dose-response study of intrafollicular injection of insulin-like growth factor-1 on follicular-fluid factors and follicle dominance in mares. Biol Reprod, 70 (4), 1063-1069. doi: 10.1095/biolreprod.103.024844

6. Ginther, O.J., Gastal, E.L., Gastal, M.O., Bergfelt, D.R., Baerwald, A.R., Pierson, R.A. (2004). Comparative Study of the Dynamics of Follicular Waves in Mares and Women. Biol Reprod, 71 (4), 1195-1201. doi: 10.1095/biolreprod.104.031054

7. Raz, T., Aharonson-Raz, K. (2012). Ovarian follicular dynamics during the estrous cycle in the mare. Israel J Vet Med, 67 (1), 11-18.

8. Acosta, T.J., Gastal, E.L., Gastal, M.O., Beg, M.A., Ginther, O.J. (2004). Differential blood flow changes between the future dominant and subordinate follicles precede diameter changes during follicle selection in mares. Biol Reprod, 71 (2), 502-507. doi: 10.1095/biolreprod.104.027896

9. Isola, J.V.V., Jessica, D Hense, J.D., Osório, C.A.P., Biswa,s S., Alberola-Ila, J., Ocañas, S.R., Schneider, A., Stout, M.B. (2024). Inflammation, immune cells, and cellular senescence in the aging ovary. Reproduction, 168 (2), e230499. doi:10.1530/REP-23-0499

10. França, D.S.., Filho, R.R.R., Alonso, M.A., Riccio, A.V., Affonso, F.J., Brito, M.M., Francischini, M.C.P., Nichi, M., Boakari, Y., Fernandes, C.B. (2025). Does age and parity affect the oxidative profile of pregnant and postpartum mares? J Equine Vet Sci, *in press*.

11. Mlodawaska, W., Grzesiak, M., Kochan, J., Nowak, A. (2018). Intrafollicular level of steroid hormones and the expression of androgen receptor in the equine ovary at puberty. Theriogenology, 121 (11), 13-20. doi:10.1016/j.theriogenology.2018.07.026

12. Satué, K., Fazio, E., Damiá, E., Barbiera, G., Medica, P., Cravana, C. (2024a). Effect of age on androgen pattern in cyclic mares. Res Vet Sci, 173 (6), 105276. doi: 10.1016/j.rvsc.2024.105276

13. Satué, K., Fazio, E., Velasco-Martínez, M.G., La Fauci, D., Barbiera, G., Medica, P., Cravana, C. (2024b). Can the reduced GH, IGH-1, and ovarian steroids concentrations be considered as suspected biomarkers of age-associated functional deficit in mares? Theriogenology, 223 (10), 75-80. doi: 10.1016/j.theriogenology.2024.08.001

14. Satué, K., Fazio, E., Velasco-Martínez, M.G., La Fauci, D., Cravana, C., Medica, P. (2024c). Effect of age on amplitude of circulating catecholamine’s change of healthy cyclic mares. Vet Res Commun, 48 (4), 2863-2868. doi:10.1007s11259-024-10443-8

15. Alyoshina, N.M., Tkachenko, M.D., Malchenko, L.A., Shmukler, Y.B., Nikishin, D.A. (2022). Uptake and metabolization of serotonin by granulosa cells form a functional barrier in the mouse ovary. Int J Mol Sci, 23 (23), 14828. doi: 10.3390/ijms232314828

16. Satué, K., Fazio, E., Ferlazzo, A., Medica, P. (2019). Intrafollicular and systemic serotonin, oestradiol and progesterone concentrations in cycling mares. Reprod Dom Anim, 54 (10), 1411-1418. doi:10.1111/rda. 13545.

17. Satué, K., Fazio, E., Medica, P., Velasco-Martinez’ M.G., Cristina Cravana’ C., Bruschetta’ G., Deborah La Fauci’ D. (2025). The pivotal interaction between 5-HT and calcium shift in pregnant Spanish Purebred mares: aging effect. Vet Sci, *in press*.

18. Satué, K., Fazio, E., Medica, P. (2023b). Estrogen-iron axis in cyclic mares: Effect of age. Theriogenology, 209 (10), 178-183. doi: 10.1016/j.theriogenology.2023.06.027

19. Satué, K., Fazio, E., La Fauci, D., Bruschetta, G., Medica P. (2023a). Adaptive response of estrogen-iron axis in pregnant Purebred Spanish mares of different age. J Equine Vet Sci, 127 (8), 104827. doi: 10.1016/j.theriogenology.2023.05.008

20. Ślebodziňski, A.B. (2005). Ovarian iodide uptake and triiodothyronine generation in follicular fluid. The enigma of the thyroid ovary interaction. Dom Anim Endocrinol, 29 (1), 97-103. doi: 10.1016/j.domaniend.2005.02.029

21. Scarler, D., Ille, N., Erd, R., Alves, B.G., Gastal, G.D.A., Paiva, S.O., Gastal, M.O., Gastal, E.L., Aurich, C. (2017). Glucocorticoid metabolism in equine follicles and oocytes. Dom Anim Endocrinol, 59 (4), 11-22. doi: 10.1016/j.domaniend.2016.10.004

Received and accepted February 4, 2025, published online March 13, 2025