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The effect of mare's age on multiple ovulation rate, embryo recovery, post-transfer pregnancy rate, and interovulatory interval in a commercial embryo transfer program in Argentina

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ABSTRACT

Advanced maternal age is an important predisposing factor on the reduction of reproductive efficiency. The aim of this study was to evaluate the effect of donor's age on several reproductive parameters in a commercial equine embryo transfer program. Donors were classified into 3 age groups: Group 1 = fillies (3 and 4 years old), Group 2 = middle age mares (aged 5–10) and Group 3 = old mares (aged 13–25). Embryo recovery, multiple ovulation and pregnancy rates and interovulatory intervals were compared amongst age groups. Group 1 (171/244, 70.1%) and Group 2 (774/1081, 71.6%) had a higher ($P < 0.005$) embryo recovery rate than Group 3 (385/701, 54.9%). Groups 2 and 3 were 2.5 and 3.4 times more likely to have multiple ovulations than Group 1 ($P < 0.05$), respectively. The effect of age group on pregnancy rate was not significant ($P > 0.05$). The interovulatory intervals length was influenced by individual mare ($P < 0.001$), age ($P < 0.04$), Day of flushing ($P = 0.009$) and by month ($P < 0.012$). The overall mean interovulatory interval of Group 1 (16.4 ± 0.17 days) and Group 2 (16.6 ± 0.12 days) was not different ($P > 0.05$), but was shorter than the one of Group 3 (17.4 ± 0.15 days; $P < 0.04$). The embryo recovery rate of flushings from Groups 1 and 2 was influenced by the length of the previous interovulatory interval ($P = 0.03$).

1. Introduction

Commercial equine embryo transfer (EET) in Argentina began in the late 1980s in polo mares. From the overall population of broodmares it has been estimated that 10–25% are aged mares (Baker et al., 1993). This relatively high percentage of aged mares might be due to the fact that genetically valuable mares usually are kept for longer in reproduction programs so that a larger number of offspring can be obtained.

Older mares have been associated with an increased interovulatory interval (IOI), resultant from a longer

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follicular phase (Carnevale et al., 1993), as well as with a lower circulating estrogen and inhibin concentrations compared to younger mares (Carnevale et al., 2002). This fact may be relevant to the practitioners in their routine clinical work. If an aged mare is going to have a longer IOI, then the chances to obtain an embryo in a breeding season would be further reduced compared with a younger mare. The IOI in the mare varies from 16 to 25 days (Ginther and Pierson, 1989), with a mean of approximately 22 days. This huge variation in IOI (16–25 days) appears to be due to the difference in the length of estrus rather than diestrus itself, which is rather constant (Ginther, 1992). Amongst other factors, this variation can be explained by breed and seasonal factors. Pony mares have, on average, a 2 days longer interval than horses (Ginther, 1992). The IOI of mares earlier in the season is longer than during the more advanced ovulatory season (summer months). This is associated with a lower mean daily LH concentration (Turner et al., 1979). During an ET program, however, the IOI of donor mares depends also on whether the mare is short-cycled with a luteolytic dose of Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or its analogs (PGF) on the day of embryo flushing. In most EET programs, PGF is administered during the same Day of flushing which varies from 7 to 10 days after ovulation. Since PGF shortens the IOI (Ginther, 1992) the Day of embryo flushing is likely to influence the subsequent IOI. Furthermore, evidence shows that the interval from PGF treatment to ovulation may influence the pregnancy rate (PR) (Cuervo-Arango and Newcombe, 2010). However, the relationship between the IOI and the subsequent embryo recovery rate (ERR) has not been investigated in donor mares during an EET programs.

Multiple ovulation rate (MO) is influenced by the age of the mare (Davies Morel et al., 2005). Older mares have a higher MO rate than younger mares (Losinno et al., 2000; Hunt et al., 2005a,b). This effect of age on MO appears to be driven by a gradual increase in the IOI and differences in gonadotropins concentrations as the mare becomes older (Carnevale et al., 1993). In addition, the interval from PGF treatment to ovulation and in turn the IOI influences the MO rate (Cuervo-Arango and Newcombe, 2010). As the interval from PGF treatment becomes longer, the MO rate increases. This effect appears to be due to an increase in the LH concentration after PGF-induced luteolysis (Ginther et al., 2008b).

Advanced maternal age is an important predisposing factor on the reduction of reproductive efficiency (Carnevale and Ginther, 1995; Carnevale et al., 2000; Hunt et al., 2005a; Carnevale, 2008). It is widely accepted that mare fertility begins to decline after 13–17 years old (y.o.) (Ginther, 1992; Losinno et al., 2000; Allen et al., 2007). A prospective cohort study (Hanlon et al., 2012) demonstrated that for each additional increase in one year of age, the first-cycle PR was reduced by a factor of 0.94 and the end-of-season PR was reduced by a factor of 0.91. Mares older than 14 years of age took longer to conceive after the start-of-mating compared with younger mares. The chances of conception for mares aged 14 years and older were 0.64 times less than mares younger than 9 y.o.

From these controlled studies, it seems clear that a large number of oocytes and resultant embryos of aged subfertile mares (with a history of reproductive failure) may be defective. However, the impact of age on embryo quality and PR has not been extensively and critically addressed in commercial EET programs (Hunt et al., 2005a).

The aim of this retrospective study was to evaluate the effect of donor's age on several reproductive parameters in a commercial EET center: embryo recovery, multiple ovulation, PR and IOI in fillies, middle age and old donor mares. It was hypothesized that: (1) ERR would be influenced by age of donor mare; (2) post-transfer PR would be lower following transfer of embryos from aged mares than from younger mares, and this difference would be more evident compared with the ERR and (3) The IOI would be longer in aged than in younger mares and would be influenced by month and Day of flushing.

2. Materials and methods

Individual reproductive records obtained from 4 consecutive breeding seasons in a commercial EET center in Argentina (33° S) were analyzed retrospectively. The embryo donor mares were Polo Argentino aged between 3 and 25 y.o. Donors were classified into 3 age groups according to the categories established by the embryo center: Group 1 = fillies (3 and 4 y.o.), Group 2 = middle age (5–10 y.o.) and Group 3 = old (13–25 y.o.). The number of mares in each age category varied amongst breeding seasons and depending on the variable analyzed (Table 1). Mares aged 11–12 were not included in the analyses so that a more clear gap in age difference was left between Groups 2 and 3. A different number of cycles (from 1 to 7) from 4 different season were used for data analyses on the effect of age on the ERR and PR (Table 1). However, only data from 2 seasons were available for determining the effect of age on the IOI and MO rate. Mares that changed age's groups during the studied period were used only once (in the younger age group).

Mares were routinely examined by transrectal palpation and ultrasonography to assess ovarian follicular activity. Once a mare reached a follicle of 32–35 mm in diameter and showed uterine edema, she was artificially

Table 1
Number of mares and observations recorded for different reproductive variables.

Age (y.o.)	Flushings[a]		Embryo transfers[b]		Ovulation rate[c]		IOI[d]	
	Mares	*n*	Mares	*n*	Mares	*n*	Mares	*n*
Fillies (3–4)	37	244	35	157	36	242	42	178
Middle age (5–10)	106	1081	100	812	98	591	95	365
Old (13–26)	40	701	38	205	37	348	49	224
Overall	183	2026	173	1174	171	1181	186	767

[a] Number of mares and flushings (*n*) performed during four breeding seasons.
[b] Number of donor mares with a positive embryo flushing and at least 1 embryo transfer (*n*) during four breeding seasons.
[c] Number of mares and cycles from each mare during two breeding seasons in which the number of ovulations per cycle were known.
[d] Number of mares with one or more interovulatory intervals (*n*) during two breeding seasons.

inseminated with fresh semen collected from 1 of the 36 stallions of proven fertility included in the ET program. Ovulation was diagnosed as the disappearance of the previous preovulatory follicle by ultrasound at 24 h intervals. The number of ovulations in each estrus was recorded. Mares were re-inseminated at 48 h intervals if ovulation had not taken place. During the 4 breeding seasons mares were inseminated with semen from a total of 36 stallions of proven fertility. These 36 stallions were used for 1–389 inseminations.

Embryo recovery was performed by uterine lavage 7–9 days after ovulation. The variation on the Day of flushing was due to the fact that no flushing was performed on Sundays. Therefore, if a mare was diagnosed as ovulated on Saturday morning, the flushing would be performed either the next Saturday (Day 7) or Monday (Day 9), otherwise all mares were flushed on Day 8. The mare's uterus was flushed with 2 L of sterile Ringer Lactate solution at 35 °C, using an equine lavage catheter CH 32 (Minitube International). The effluent was collected through a sterile plastic closed circuit, connected to an embryo filter. Then, the filter was transferred to the laboratory protected with a neoprene cover. The content was rinsed into a sterile petri dish, where embryos were searched using a stereomicroscope at 20×. The number of embryos recovered was recorded for each flush. A uterine flushing was regarded as positive when one or more embryos were found.

Embryos were washed 3 times with Ringer Lactate at 35 °C under a laminar flow cabin, classified by developmental stage (morula, early blastocyst, blastocyst, and expanded blastocyst) and morphology, according to the scoring system proposed by McKinnon and Squires (1988). Then, the embryos were placed on a plate with equine embryo holding (Syngro® Bioniche Animal Health USA) for a maximum of 1 h at room temperature. Then, embryos were loaded on 0.5 ml sterile straws in an EET disposable pipette and transvaginal transfer from the same operator to a synchronized recipient mare, which had not received any treatments pre- or post-transfer.

Following uterine flushing, each donor was treated with 250 μg of cloprostenol (DL cloprostenol, 250 μg/ml, Estrumate, MSD, Argentina) to induce luteolysis. A total of 2026 flushings from 4 consecutive breeding seasons (244 from fillies, 1081 from young mares, and 701 from old mares) were analyzed for determining the overall effect of donor's age on the ERR.

Pregnancy diagnosis was performed by ultrasound 7–10 days after embryo transfer. The number of mares and embryo transfers from each age group is shown in Table 1.

The IOI was described as the interval in days between two consecutive ovulations. A total of 178 IOI from Group 1 ($n = 42$ filly donors), 365 from Group 2 ($n = 95$ middle age donors) and 224 from Group 3 ($n = 49$ old donors) from 2 breeding seasons were available for analysis (Table 1). The MO rate was calculated by dividing the number of cycles with 2 or more ovulation by the total number of estrous cycles. Overall, 242 cycles from fillies ($n = 36$), 591 from middle age mares ($n = 98$) and 348 from old mares ($n = 37$) were available from two breeding seasons for determining the effect of age on MO.

2.1. Statistical analysis

Embryo recovery and MO rates were analyzed by PROC GENMOD procedure of SAS V9.3 using a binomial distribution. The effect of age on MO and PR were calculated by a binomial distribution with a model that also considered the effect of breeding season. The effect of age on the ERR was calculated by a binary logistic regression. The model included the stallion, breeding season and donor's age as independent categorical variables. The stallion and age variables were crossed to test any interactions amongst them. Only individual stallions with more than 50 flushings were considered ($n = 11$). Data from the rest ($n = 25$) were pooled and considered as one.

The effect of mare's age, individual mare, Day of flushing, month and season on the length of the IOI was determined by a General Linear Model (GLM) of variance. The model included as dependent variable the IOI in days, and as independent variables the age group (3 levels: fillies, middle age and old mares), the mare effect, the Day of flushing (3 levels: Day 7, 8 and 9 after ovulation), month in which the flushing took place (7 levels: October, November, December, January, February, March and April) and season (1 and 2). For comparisons amongst levels, a Tukey's range (Post hoc analysis) test was used. The IOI data were not normally distributed and so were ranked for GLM analysis. Overall this analysis studied 767 IOI from 186 mares. Furthermore, a GLM with a repeated statement to account for autocorrelation between sequential observations (consecutive IOI from the same mare) was performed to test the effect of repeated measurements within individuals of different ages. In this analysis 6 fillies, 9 middle age mares and 11 old mares had at least four consecutive IOI during the same breeding season with flushings performed on the same day (Day 8) and therefore were eligible for repeated measurement analysis. The first IOI of each mare used in this analysis took place during October.

The relationship between the length of IOI (in groups of 2-days difference), the age of the donor mare and the ERR was determined by binary logistic regression. The model included also the stallion as an independent variable. The ERR result was obtained from the flushing subsequent to the previous IOI.

3. Results

3.1. Embryo recovery rate

Data on the exact Day of flushing were available for 1207 flushings from 2 breeding seasons (105, 1023 and 79 flushings for Day 7, 8 and 9, respectively). Most embryo flushes were performed on Day 8 (84.8%). More old mares were flushed on Day 9 (14.4%) compared to fillies (1.3%) or middle age mares (4.1%).

There was a significant effect ($P < 0.001$) of age group on ERR. This effect was not dependent on breeding season ($P > 0.05$). In addition, the stallion had an effect ($P < 0.001$) on the ERR (Table 2). Both fillies (171/244, 70.1%) and middle age mares (774/1081, 71.6%) had a higher ($P < 0.005$) ERR than old mares (385/701, 54.9%). The ERR for flushings on Day 7, 8 or 9 was not different in young mares (fillies and

Table 2
Effect of stallion and donor's age on embryo recovery rate.

Stallion	Fillies (3–4)		Middle age (5–10)		Old (13–26)		Overall	
	+flushings/*n*	ERR %	+flushings/*n*	ERR %	+flushings/*n*	ERR %		
1	8/9	88.8	41/50	82.0	31/47	65.9	80/106	75.5[a]
2	23/27	85.2	31/41	75.6	10/18	55.5	64/86	74.4[a]
3	7/8	87.5	70/93	75.2	30/46	65.2	107/147	72.8[a]
4	4/10	40.0	14/40	35.0	15/43	34.9	33/93	35.5[d]
5	23/33	69.7	124/165	75.1	61/116	52.6	208/314	66.2[ab]
6	3/3	100.0	26/35	74.2	11/18	61.1	40/56	71.4[a]
7	39/51	76.5	144/178	80.9	99/160	61.8	282/389	72.5[a]
8	8/14	57.1	85/142	59.8	21/40	52.5	114/196	58.1[c]
9	6/11	54.5	21/32	65.6	3/8	37.5	30/51	58.8[c]
10	41/64	64.0	143/194	73.7	56/129	43.4	240/387	62.0[bc]
11	9/14	64.2	57/69	82.6	40/56	71.4	106/139	76.3[a]
12–36	–	–	18/42	42.9	8/20	40.0	26/62	41.9[d]
Overall	171/244	70.1[a]	774/1081	71.6[a]	385/701	54.9[b]	1330/2026	65.6

+flushing/*n*: number of positive flushings/total number of flushings obtained from 4 consecutive breeding seasons. The binary logistic regression analysis showed a significant effect of stallion ($P < 0.001$) and donor mare's age ($P < 0.001$), but no interaction ($P > 0.05$) between stallion and donor's age on the embryo recovery rate (ERR). Within stallions and age groups, different letters (a, b, c) indicate a significant ($P < 0.05$) difference in the ERR. Only stallions with more than 50 inseminations (cycles) were analyzed individually ($n = 11$). The data from rest ($n = 25$) were pooled and analyzed as one.

Table 3
Effect of embryo donor's age on MO, ERR and post-transfer PR.

Donor's age group	Age (y.o.)	MO rate (%)	ERR/day of flushing (%)				Post-transfer PR (%)
			Day 7	Day 8	Day 9	All days	
Fillies	3–4	39/242	36/54	126/173	2/3	164/230	115/157
		16.1[a]	66.7	72.8	66.6	71.3[a]	73.2
Middle age	5–10	197/591	31/43	412/562	16/26	459/631	592/812
		33.3[b]	72.1	73.3	61.5	72.7[a]	72.9
Old	13–26	149/348	5/8	162/288	15/50	182/346	140/205
		42.8[c]	62.5[#]	56.2[#]	30.0[‡]	52.6[b]	68.3

All data were obtained from two different breeding seasons. Within column, different superscript (a, b, c), indicate a significant difference ($P < 0.05$). Within age group (row), different superscripts (#, ‡) indicate a significant difference in ERR (embryo recovery rate; $P = 0.001$).

middle age mares; $P > 0.05$). However, in old mares the ERR from flushings on Day 7 (62.5%) and 8 (56.2%) was higher ($P = 0.001$) than the ERR on Day 9 (30.0%; Table 3).

3.2. Post-transfer pregnancy rates

The effect of age group on post-transfer PR was not significant ($P > 0.05$; Table 2).

3.3. Ovulation rate

Overall, the MO rate was 32.6% and was influenced by age ($P < 0.005$). The MO rate increased as the mares became older (Table 2). Middle age mares were 2.5 times more likely (33.3%) to have MO than fillies (16.1%; $P < 0.05$; OR = 2.5). Old mares were 3.4 times more likely to have MO (42.8%) than fillies (OR = 3.42) (Table 2). No effect was detected amongst different years ($P > 0.05$).

3.4. Interovulatory interval

The IOI varied from 11 to 25 days. The IOI length was influenced by age of donor mare ($P = 0.04$; Fig. 1), day of flushing ($P = 0.009$; Fig. 2), individual mare ($P < 0.001$) and by month ($P = 0.012$; Fig. 1). The overall mean IOI of fillies (16.4 ± 0.17 days) and middle age mares (16.6 ± 0.12 days) was not different ($P > 0.05$; Fig. 1), but was shorter than the

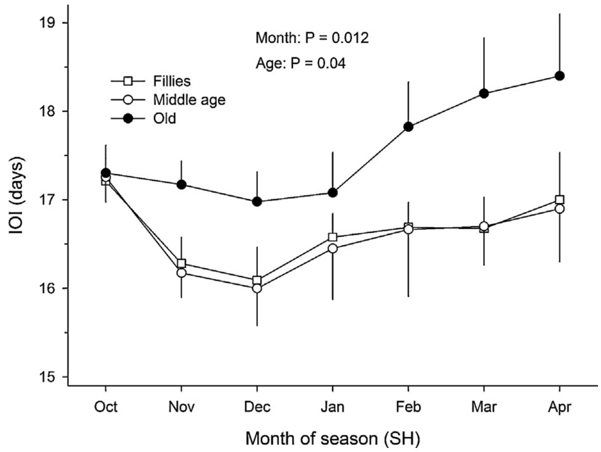

Fig. 1. Mean inter-ovulatory interval (IOI) ± S.E.M. of fillies ($n = 42$ mares and 178 IOIs), middle age ($n = 95$ mares and 365 IOIs) and old mares ($n = 49$ mares and 224 IOIs) arranged by month (Southern hemisphere) from two breeding seasons. The General Linear Model of variance found a significant effect of age ($P = 0.04$) and of month ($P = 0.012$) on the length of the IOI.

IOI of old mares (17.4 ± 0.15 days; $P = 0.04$). The mean IOI of mares flushed on Day 7, 8 and 9 was 16.1 ± 0.29 (range 11–22 days, median of 16 days), 16.8 ± 0.09 (range 11–25 days, median 17 days) and 17.5 ± 0.33 days (range 11–25 days, median of 17 days), respectively (Fig. 2). There was no

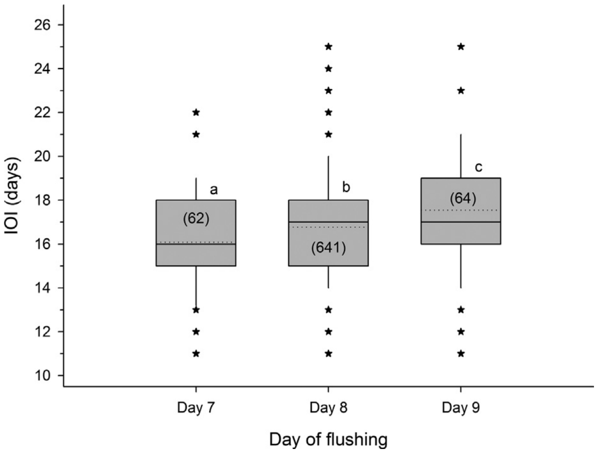

Fig. 2. Box plot distribution of inter-ovulatory intervals (IOI) of mares flushed on Day 7 ($n = 62$), Day 8 ($n = 641$) and Day 9 ($n = 64$). All mares were short-cycled with PGF immediately after the uterine flushing. Different letters (a, b, c) on the box of each Day indicate significant difference ($P < 0.05$) in the length of the IOI.

effect of breeding season on the length of the IOI ($P > 0.1$) or significant interaction between the age of mare and day of flushing on the IOI length ($P > 0.1$).

In the analysis for repeated IOI measurement for each mare, there was no effect of number of consecutive IOI on the length of IOI ($P > 0.1$), but there was a tendency of age to influence the length of the IOI ($P = 0.1$). The interaction between age and number of consecutive IOI approached significance ($P = 0.06$). This interaction resulted from a less pronounced reduction in the IOI length as the season advanced in old mares compared with younger mares (Fig. 3).

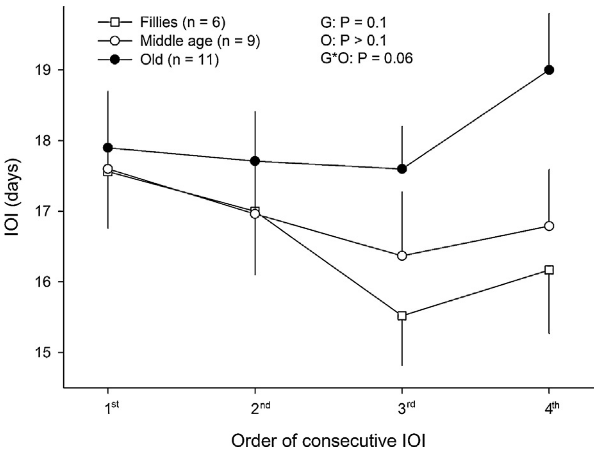

Fig. 3. Mean inter-ovulatory interval (IOI) ± S.E.M. of fillies ($n = 6$ mares and 24 IOIs), middle age ($n = 9$ mares and 36 IOIs) and old mares ($n = 11$ mares and 44 IOIs) arranged by consecutive order of IOI from 1 breeding season. Only mares with 4 consecutive IOI and flushings performed 8 days after ovulation were included in the analysis. The first IOI (O = 1st) of each mare took place in October (Southern hemisphere). The repeated measurement of sequential observations analysis showed a tendency of donor's age to influence the length of the IOI (G: $P = 0.1$). The order of consecutive IOI had no effect on its length (O: $P > 0.1$). There was an interaction effect (G*O: $P = 0.06$) between the donor's age and the order of consecutive IOI on the IOI length.

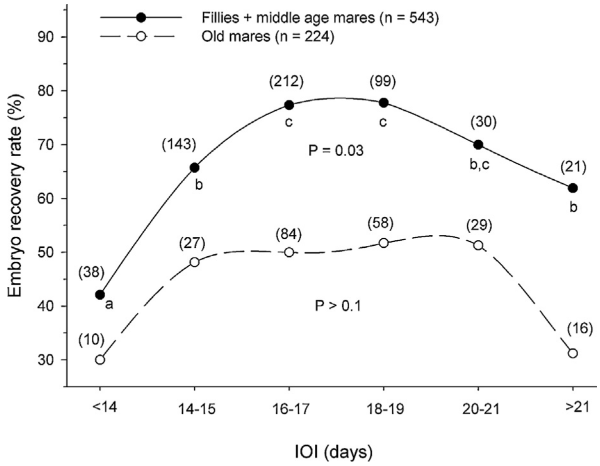

Fig. 4. Relationship between the previous interovulatory interval (IOI) and the embryo recovery rate (ERR) of young (aged 3–10) and old donor mares (aged > 12) flushed 7, 8 and 9 days post-ovulation during two breeding seasons. The IOI was grouped in two-day intervals. A binary logistic regression showed a significant effect of the IOI on the ERR for young mares ($P = 0.03$) but not for old mares ($P > 0.1$). The number of IOI for each two-day group is shown in brackets. There was no interaction effect ($P > 0.1$) between stallion and the IOI length on the ERR.

The ERR of flushings from fillies and middle age donor mares was influenced by the length of the previous IOI ($P = 0.03$; Fig. 4). There was no interaction between stallion and length of IOI on the ERR ($P > 0.1$). The ERR increased gradually as the IOI became longer from 12 to 17 days, to decrease slightly when the IOI was >21 days. This negative correlation between ERR and IOI was not observed in old mares ($P > 0.1$, Fig. 4).

4. Discussion

The hypothesis 1 that the ERR would be influenced by age of donor mare was supported by the results of this study. The ERR was lower in older mares compared to Groups 1 and 2 (younger mares). There was no difference in ERR between the young and middle age groups, which is consistent with the reported reduction in the quality of oocytes and reproductive parameters that occurs after 12–13 years of age (reviewed by Vanderwall, 2008). This difference agrees with the results reported by others (Losinno et al., 2008). It is suggested that the presence of intrinsic defects of oocytes and/or embryos significantly contributes to a reduction in embryonic survival related to maternal age (Vanderwall, 2008). Possible causes of structural and/or functional alterations in oocytes and competence in embryonic development, associated with the decline in age-related female fertility are: a decrease in mitochondrial activity and an increased incidence of chromosome abnormalities (Losinno, 2006; Rambags, 2007). This is further confirmed by the finding of the present study in which the ERR of old mares flushed on Day 8 was significantly higher than the ERR of Day 9 flushings from the same age group. This difference could account for an increase in the number of embryo losses between Day 8 and 9 in old mares.

Hypothesis 2 that post-transfer PR would be lower following transfer of embryos from aged mares than from younger mares was not substantiated by the results of this study. In contrast with previous reports, the present results showed no significant differences in PR 15 days after ET between aged and younger mares (Pickett et al., 1987; Vogelsang and Vogelsang, 1989; Squires et al., 1999, 2003; Stout, 2006; Losinno et al., 2008; Mortensen et al., 2009; Atwood and Bowen, 2011; Pessoa et al., 2011). This could be explained, at least in part, because of a vast majority of equine embryos recovered in the present study were of good morphological quality (>90% grade 1 or 2). Furthermore, it is expected that embryos that degenerate during early development are unable to complete oviductal transit and are not recovered. In this regard, the PR of Day 4 embryos collected from the oviduct of aged subfertile mares did yield a lower PR than early embryos of young mares (Ball et al., 1989). Perhaps in the latter study, if embryo recovery would have been delayed for an extra 5 days (Day 9), the subsequent transfer might have yielded a higher PR.

The MO rate of different age groups of the present study are consistent with data reported by other authors (Davies Morel et al., 2005; Losinno et al., 2008) who concluded that aging was associated with an increased incidence of MO. A recent study (Panzani et al., 2014) demonstrated that the ovulation rate was significantly higher in mares aged between 16 and 20 y.o. compared with other age groups of mares. It is accepted that the incidence of MO increases along with age (Davies Morel et al., 2005). In this line, the present results show a gradual increase in MO amongst increasing age groups. Other reports demonstrated that MO rate is higher in Thoroughbred mares than in Quarter horse and/or Arabian mares (Dimmick et al., 1993; Vivo and Vinuesa, 1993; Newcombe, 1994). This could explain the high incidence of MO in the young mares; since most of high performance Polo mares are genetically originated from Thoroughbred.

The hypothesis 3 that the IOI would be longer in aged than in younger mares and would be influenced by month and Day of flushing was supported by the results of this study. The analysis of the IOI revealed no differences between the two groups of younger mares, but there were small significant differences (approximately 1 day longer on average) between these two groups and the older mares, which is consistent with that reported by other authors (Carnevale et al., 1993, 1994; Ginther et al., 2008a). The lengthening of the estrous cycle in old mares could be caused by a slower growth rate of the dominant follicle than in young mares (Rambags, 2007; Altermatt et al., 2012). It has also been observed that old mares have fewer ovarian follicles and lower concentrations of LH during the preovulatory surge (Ginther et al., 2009). In addition, the IOI was longer at the beginning and end of the season, as it has been reported previously (Ginther and Pierson, 1989; Ginther, 1992). The effect of month was even more evident in older mares, with the longest IOI occurring during the autumn months.

The repeated measurement analysis of individual mares during four consecutive IOI did not show a significant effect of time (number of consecutive IOI) on the IOI length as opposed to the effect of month. This discrepancy might be explained, at least in part, by the decreased sample size of mares used in the repeated measurement analysis due to the reduced availability of mares with at least four consecutive IOI with Day 8 flushings, compared with the overall analysis. Furthermore, many of these mares had two IOI within the same month, and overall the four IOI took place during two to three consecutive months, which is probably not enough time to make a significant difference in the hours of daylight.

According to the results of this study, it seems that the main factor influencing the IOI is the mare itself, that is, the individual variation. Since the data obtained from two breeding seasons involved several IOI from the same mare (average of 4.2 IOIs per mare, range 1–7 IOIs), the GLM analysis showed differences amongst mares in the mean IOI length of more than 10 days (between 13 and 25 days). Mares with short intervals are usually found with a large follicle (>30 mm) at the time of flushing (time of PGF treatment), whereas mares with long IOI have small follicles at the time of flushing which may take 10–16 days to grow and ovulate or on fewer cases may have a large follicle which does not ovulate but regresses. It has been shown that there is a good degree of individual repeatability in the preovulatory follicular diameter (Cuervo-Arango and Newcombe, 2008) and in the length of the IOI within consecutive cycles of individual mares (reviewed by Ginther, 1992).

An interesting observation was to find a negative correlation between the ERR and the IOI. As the IOI was shorter, the ERR was reduced. A similar relationship has been reported in Thoroughbred mares (Cuervo-Arango and Newcombe, 2010). In the latter study, the interval from PGF treatment to ovulation also influenced the pregnancy outcome of the mating of the subsequent estrus. However, the reason why this effect was not observed in older mares is unknown and requires further research to elucidate the exact reason.

In summary, according to this study, in a particular breed of mares and in a large scale EET commercial program, the age of the donor mare affected ERR, the incidence of MO and the IOI, but not the post-transfer PR. Furthermore, the IOI length influenced the outcome of the subsequent flushing. This effect was observed in fillies and young mares only. The conclusions of this study may be relevant for the veterinarian in order to learn how likely an aged mare is to perform during an EET program.

Conflict of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the present paper.

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