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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.