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Abstract:
Embryonic vesicle growth in the mare is easily monitored by ultrasound. Apart from pregnancy diagnosis, assessment of the embryonic vesicle in practice is also useful to evaluate its viability. Although subjected to individual variation, embryo growth rate follows a constant pattern in the early stages of development in relation to embryonic age. Previous studies have shown a significant effect of some factors routinely used in practice, such as post-ovulation insemination and embryo transfer, on embryonic growth and the time in which the vesicle is first detected. This study attempts to confirm previous results in different settings and characterise the reasons for this delay in growth. A total of 159 pregnancies from different mating protocols: 1) pre-ovulation natural mating, 2) pre-ovulation natural mating and transfer into recipient mares, 3) post-ovulation natural mating, and 4) post-ovulation AI with frozen spermatozoa were evaluated ultrasonographically from day 12 to 19 of pregnancy and vesicle diameters recorded. Regression analysis for vesicle diameter-embryonic age was performed for each group and mean vesicle diameter at different

age periods among groups were tested for statistical difference with a general linear model of variance. There was no significant difference between groups 1 and 2 ($P = 0.73$) or between groups 3 and 4 ($P = 0.71$). However both pre-ovulation groups (1 and 2) had larger vesicle diameters ($P < 0.000$) at any embryonic age analysed than either post-ovulation group (3 and 4). In conclusion, post-ovulation inseminations produced pregnancies with smaller vesicle diameters equivalent to approximately one day's growth.

Effect of type of semen, time of insemination relative to ovulation and embryo transfer on early equine embryonic vesicle growth as determined by ultrasound

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Abstract

Embryonic vesicle growth in the mare is easily monitored by ultrasound. Apart from pregnancy diagnosis, assessment of the embryonic vesicle in practice is also useful to evaluate its viability. Although subjected to individual variation, embryo growth rate follows a constant pattern in the early stages of development in relation to embryonic age. Previous studies have shown a significant effect of some factors routinely used in practice, such as post-ovulation insemination and embryo transfer, on embryonic growth and the time in which the vesicle is first detected. This study attempts to confirm previous results in different settings and characterise the reasons for this delay in growth. A total of 159 pregnancies from different mating protocols: 1) pre-ovulation natural mating, 2) pre-ovulation natural mating and transfer into recipient mares, 3) post-ovulation natural mating, and 4) post-ovulation AI with frozen spermatozoa were evaluated ultrasonographically from day 12 to 19 of pregnancy and vesicle diameters recorded. Regression analysis for vesicle diameter-embryonic age was performed for each group and mean vesicle diameter at different age periods among groups were tested for statistical difference with a general linear model of variance. There was no significant difference between groups 1 and 2 ($P = 0.73$) or between groups 3 and 4 ($P = 0.71$). However both pre-ovulation groups (1 and 2) had larger vesicle diameters ($P < 0.000$) at any embryonic age analysed than

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Keywords: Mare; Embryonic vesicle diameter; Insemination; Ovulation; Embryo transfer

1. Introduction

The horse embryo is conceived in the ampulla, near the junction with the isthmus where is retained until the beginning of oviductal descent approximately 4 days post-fertilization [1] and culminates when the embryo enters the uterus sometime between 6 and 6.5 days after ovulation [2,3] During the oviductal stage, the embryo does not increase its size remaining no different from the original unfertilized oocyte with a diameter range from 149 to 178 μm [2]. At the uterine stage the conceptus begins a rapid growth phase in which the vesicle diameter increases gradually maintaining its spherical shape until about 17 to 18 days post-ovulation when it reaches a plateau and the vesicle outline becomes irregular as it intimates with the endometrial folds.

Monitoring of conceptus diameter is easily and routinely performed in practice by transrectal ultrasonography. Although the minimum vesicle size visible with the scanner would depend on the probe resolution and experience of the operator, in most occasions vesicles of 3 to 4 mm can be accurately identified. The mean day of first detection of embryonic vesicles has been reported to be 11.1 days post-ovulation with a mean vesicle diameter of 6.7 mm [4]. Earlier assessment of vesicle diameter is also possible by measurement of embryos rematinged by uterine flushing at known stages of development. Several factors affecting the early embryonic vesicle diameter have been proposed: time of insemination relative to ovulation [4], number and synchrony of multiple ovulations [5], presence of multiple vesicles in the uterus [5-6-7], degree of

synchrony donor-recipient in embryo transfer technology [8], health of uterus (Newcombe and Cuervo-Arango, unpublished) and age of mare and oocyte quality [9]. Knowledge of embryonic vesicle diameter in relation to ovulation date during earlier stages and shape and position of the embryo proper within the conceptus thereafter may have clinical relevance for the practitioner since impending embryonic failure can be suspected in small for age vesicles [7] and in conceptuses embryo proper's abnormal development [10].

Embryonic age is either determined from the point in which ovulation was first detected (in pre-ovulation inseminations) or from the point of insemination (in post-ovulation inseminations). Accuracy of determining the embryonic age would depend on the frequency of ultrasound examinations to detect ovulation (pre-ovulation inseminations) or on the assumption that fertilization occurs soon after insemination (post-ovulation inseminations).

In pre-ovulation inseminations equine sperm is known to reach the oviducts as early as 0.5 h post-insemination [11], however it has been proven that sperm located in the uterine lumen between 2.5 and 4 h post-insemination is still involved in fertilization since uterine lavage with large volume saline 2.5 h but not 4 h after insemination, decreased pregnancy rates significantly [12,13]. The time taken for the oocyte to reach the fertilization site is assumed to be short like in other domestic species such as the pig in which the oocytes take about 30 to 45 min [14,15]. Therefore it could be assumed that fertilization after post-ovulation insemination would occur somewhere at or after 4 h post-insemination and yet difference in vesicle diameter equivalent to one day's growth was found between mares inseminated before and after ovulation [4]. In the latter study however, embryonic ages of the pre-ovulation group were known only to with ± 12 h accuracy. The authors concluded that the difference in growth could be

attributed, at least in part, to requirements for sperm capacitation in the post-ovulation group. If the reason for this delay were the requirements for sperm capacitation, it could be hypothesised that post-ovulation insemination with frozen semen (which after thawing acquires a capacitation-like status [16]), would fertilize the oocyte faster than fresh spermatozoa.

The objectives of this study were to determine a) the effect of different types of semen (frozen and fresh) on the time taken from insemination to fertilization and subsequent embryonic development and b) the effect of embryo transfer on early embryonic vesicle development. It was hypothesised that fertilization after insemination with frozen semen would occur faster than that with fresh semen in post-ovulation inseminations and that the procedure of embryo handling and asynchrony of donor-recipient would retard post-transfer embryonic vesicle growth.

2. Materials and methods

2.1. Animals and ultrasound measurements

Embryonic vesicle diameters in mares of various breeds (Irish draught, Warmblood and Thoroughbred) resident in a fertility veterinary clinic were evaluated by ultrasonography with a 7.5 MHz linear probe during the 2007 and 2008 breeding seasons. The vesicle diameter was obtained from average of two linear measurements of the conceptus taken at right angles when the image of the vesicle was maximum using the electronic callipers.

2.2. Experimental design

Different mating protocols were evaluated to study the relationship between embryonic age and vesicle diameter. The mating protocols were classified into one of the following four groups:

1. **Pre-ovulation mating with fresh semen:** A total of 42 mares were mated naturally. Subsequently the mares were examined by transrectal ultrasound every 8 h until detection of ovulation. Embryonic age was estimated from the mid point of the period in which ovulation was first detected. Therefore the time of ovulation was known with ± 4 h accuracy. Vesicle diameters of the 42 pregnancies were measured in 96 occasions.

2. **Pre-ovulation mating with fresh semen and subsequent embryo transfer into recipient mares**: A total of 39 mares were mated naturally before ovulation. As in group 1, mares were examined every 8 hours until detection of ovulation. Donor mares were flushed 7 to 8 days post ovulation and recovered embryos transferred non-surgically into recipient mares. Recipient mares had ovulated 1 to 3 days after the donor (median synchrony of 1.5 days behind) and had no treatment at or after embryo transfer which took place within 30 minutes of flushing. Embryonic age was estimated as in group 1. A total of 39 embryonic vesicles were measured in 91 occasions were recorded.
3. **Post-ovulation mating with fresh semen**: A total of 35 mares were mated naturally after ovulation had been detected. All mares were mated within 24 h of ovulation. Embryonic age was estimated from the time of insemination to the point when vesicle diameter was measured by ultrasound. A total of 100 measurements of vesicle diameter from 35 pregnancies were recorded.

4. **Post-ovulation insemination with frozen semen**: A total of 43 mares were artificially inseminated into the uterine body with frozen/thawed semen 0 to 8 h post ovulation. Embryonic age was estimated from the time of insemination to the point when vesicle diameter was measured by ultrasound. A total of 70 measurements of vesicle diameter from 43 pregnancies were recorded.

In all groups, pregnancies were from mares which had only single ovulations and with single embryos which were considered to be normal and were known to be viable up to at least 40 days. Embryonic vesicle diameter measurements were taken approximately from day 11 to 19 of pregnancy in mares from all groups. Mares with embryonic vesicles recorded more than once were examined at different time periods. Although intervals between examinations ranged from 24 to 72 h, in most occasions embryonic vesicle from same mares were scanned at 24 h-period all made in the morning between 9 and 11 am.

Daily embryonic vesicle growth rates were calculated for the pre-ovulation and post-ovulation groups with data from mares with two consecutive vesicle diameter readings made 24 h apart (n = 40 and 41 pregnancies respectively).

2.3. Statistical analysis

Age-diameter pairs for each group were scatter plotted to calculate quadratic regression fits of growth rates for each group. In order to test statistically the difference in vesicle diameters among groups, a general linear model of variance (Minitab 15®) was performed. The model was formed by the embryonic vesicle diameters (response) and by the four groups and the following time-periods: 300, 316, 324, 336, 360, 372 and 384 h (blocks). In addition, pooled data from groups 1 and 2 (pre-ovulation insemination) were also tested at the same time periods with pooled data from groups 3

and 4 (post-ovulation insemination). Tukey's comparison test was used to compare any difference among groups and time periods.

3. Result

Regression curves of embryonic vesicle diameters of groups 1 and 2 as well as of groups 3 and 4 did not visibly differ respectively during the recorded period as shown in **Fig. 1**. Regression fits of vesicle diameters from pooled data of pre-ovulation and post-ovulation groups showed however a clear discrepancy in size equivalent to approximately one day's growth (**Fig.2**). Vesicle diameters of both pre-ovulation insemination groups were not significantly different ($P = 0.73$). In the same way, post-ovulation inseminations with fresh or frozen semen did not produce different embryonic vesicle diameters ($P = 0.71$). Transferred (group 2) and non-transferred (group 1) embryonic vesicles conceived from pre-ovulation inseminations were significantly larger than either post-ovulation group, with frozen ($P < 0.000$) or fresh semen ($P < 0.000$). Mean vesicle diameter for each group at each time period is shown **Table 1**.

When data from both pre-ovulation and post-ovulation inseminations were pooled together, overall mean vesicle diameter of pre-ovulation embryos was 3.7 ± 0.7 mm larger than that of post-ovulation groups (**Table 2**). Daily embryonic vesicle growth rates for pre-ovulation and post-ovulation groups differ significantly only on day 12 of pregnancy. Daily growth rates for days 12 to 17 for embryonic vesicles of pre- and post-ovulation groups are shown in **Table 3**.

4. Discussion

This study focused on the effect of time of insemination relative to ovulation, type of semen used and effect of embryo handling and transfer on early embryonic vesicle

diameter and growth measured by ultrasonographic examination. The results showed that the only factor affecting the vesicle diameter at a particular known age of pregnancy was whether spermatozoa were present in the oviducts at the time of ovulation. Neither embryo transfer nor type of semen (fresh versus frozen) had a significant effect on embryo size in pre- or post-ovulation inseminations respectively.

Regression curves of pooled data of vesicle diameters from pre- and post-ovulations groups showed a clear pattern in which embryos conceived from post-ovulation inseminations needed approximately one day longer to attain vesicle diameters similar to those of the pre-ovulation group.

Daily embryonic vesicle growth rates for the pre- and post-ovulation groups did only differ significantly (**Table 3**) on day 12 of pregnancy. Daily growth pattern in the pre-ovulation group increased gradually from day 12 to 14 from which underwent a decline until it reached a plateau on days 17 to 18 (**Table 3; Fig. 2**). The reason for the discrepancy in daily growth rate between both groups seems clear when the daily growth means of the post-ovulation group (**Table 3)** are advanced one day to match the similar patter of the pre-ovulation group. This leads to the suggestion that embryos conceived from post-ovulation insemination have similar daily growth patterns to those from pre-ovulation inseminations but enter the uterus initiating the rapid growth one day later.

Several theories could be suggested to explain this delay equivalent to one day's growth: because the growth rate and viability of vesicles from both groups were not different, it appears that fertilization and embryo development in pregnancies from pre- and post-ovulation inseminations occurred normally. A logical thought would be that fertilization occurs later in post-ovulation inseminations either because of delayed sperm transport to the fertilization site or longer time required for spermatozoa to

205 capacitate. On the other hand, and due to the fact that embryo growth is not initiated
206 until the long oviductal descent is completed, another attractive suggestion would be to
207 propose a longer oviductal descent of oocytes fertilized from post-ovulation
208 inseminations. Along with data reviewed from the literature, in the equine and other
209 species, and results obtained in this study, the authors will discuss the feasibility of the
210 latter theories in an attempt to explain the reason(s) for this discrepancy in size.
211
212 *4.1. Spermatozoa transport and release from sperm reservoirs*
213 Equine spermatozoa, either introduced by natural mating or artificial insemination, are
214 deposited in the uterine lumen. From there semen is carried rapidly towards the oviducts
215 powered by uterine contractions which propel sperm back and forth within the uterine
216 lumen [17]. Some of these spermatozoa gain access to the oviducts as early as 0.5 h
217 regardless the site of the preovulatory follicle [11]. Some more extra time must be
218 needed for all spermatozoa involved in fertilization to reach the oviducts since uterine
219 lavage with large volume of saline 2.5 h after insemination significantly lowered
220 pregnancy rates [12]. As in other species, but especially in the mare, the female
221 reproductive tract's environment is able to store and maintain viable spermatozoa for
222 long periods of time (up to 7 days in the horse, [4,18]; 30 h in the pig, [19]) and,
223 presumably, activating them at a given time [20]. The places where spermatozoa are
224 located are the sperm reservoirs which can be found mainly in the oviductal isthmus
225 [21,22] and perhaps in some uterine glands in the mare [11]. Several factors are thought
226 to explain the formation of sperm reservoirs in the pig, including the narrowed lumen,
227 viscous mucus, lower temperature, local enzymatic and ionic milieu, selective binding
228 of spermatozoa to the epithelium, and specific tubal fluid components, all of which
229 primarily lead to sperm quiescence, reviewed by [20].

The latter studies discuss timing of sperm transport and storage within the uterine lumen. Nevertheless, all these experiments have been carried out during the pre-ovulatory stage which could be different from the post-ovulatory period in terms of tubal environment. In fact, insemination trials in post-ovulated sows found no sperm 5 to 6 h after AI in the utero-tubal junction in any of the sows inseminated (30 h post-ovulation) and only in 25 % of those inseminated 18 h post-ovulation [23]. Authors of the latter and different studies in the same species concluded that sperm transport during the post-ovulation period is impaired [24,25]. These post-ovulated sows appeared to have a lowered uterine contraction rate as time from ovulation increased. This however may not apply to mares inseminated soon after ovulation, like in the frozen post-ovulation group in which all mares were inseminated 0 to 8 h post-ovulation. In the equine species the oocyte remains viable for longer than in the sow with viable pregnancies up to 24 to 30 h post ovulation [4], (Newcombe, unpublished) in contrast to 8 to 16 h in the sow [25] Sows ovulated 18 to 30 h appear to be far away from the physiological stage required for mating.

Elegant studies in swine have also proved that the phenomenon of follicular collapse and ovulation with the release of follicular fluid rich in progesterone and many other active components is essential for the release of spermatozoa from the sperm reservoirs and fertilization [26,27]. In these studies, microinjections of exogenous progesterone or progesterone-rich follicular fluid under the serosal layer of the oviduct surrounding the sperm reservoirs or directly into the sperm reservoir provoked a prominent release of spermatozoa and a 34 % incidence of polyspermy vs 2 % in controls. Progesterone concentrations in the follicular fluid of this species are 100 to 1000 times higher than in peripheral blood [28]. Similar to sow's follicular fluid, mare's has increasing concentration of progesterone as ovulation approaches [29].

If swine studies were extrapolated to the horse it could be suggested that one of the reason for the discrepancy in embryonic size between the pre- and post-ovulation groups is longer time for the spermatozoa to reach and fertilize the oocyte.

4.2. Sperm capacitation

One of the objectives of the present study was to determine whether "pre-capacitated" spermatozoa as it is the case of frozen/thaw sperm [16] was able to fertilize the oocyte faster than fresh semen when inseminated post-ovulation. Capacitation is regarded as the destabilizing process, particularly related to the apical sperm membrane necessary for fertilization, which is driven by changes in the oviductal fluid especially in bicarbonate concentrations [30,31]. Since the process of capacitation is triggered when spermatozoa are released from sperm reservoirs as it moves to proximal parts of the oviduct with fluid which is richer in bicarbonate [20], it would be an attractive theory to explain, at least in part, the delay in fertilization after post-ovulation insemination as it was once suggested two decades ago [4]. We however have found no significant difference in vesicle diameters between post-ovulation inseminations with fresh and frozen semen. This lack of difference could be due to the relative small number of mares used in the study and large individual variation in vesicle diameters which would not allow identifying a small difference in size (e.g. 4 to 8 h delay). The other possible reason would be that frozen/thawed spermatozoa still requires oviductal environment changes to complete the capacitation process.

4.3. Embryo's oviductal descent

The horse embryo is unusual with respect to other domestic species in terms of time spent in the oviduct before entering the uterus as an early blastocyst. During the

280 oviductal stage (6 to 6.5 days) the embryo keeps its original size [3], and is not until the
281 embryo is able to move freely along the uterine lumen when the blastocyst expands
282 beginning its rapid growth.
283 Unlike in other species, there is a lack of knowledge on the oviductal fluid components
284 and hormonal milieu required for the nourishment and early development of the equine
285 embryo [32]. And although, it appears that the embryo itself is the one that makes its
286 way to the utero-tubal junction by the local effect of embryonic prostaglandins [33], it
287 could be hypothesised that changes in oviductal environment and hormonal milieu
288 (estrogens/progesterone ratio) in a more advanced post-ovulatory stage would delay, at
289 least in part, the oviductal descent of embryos conceived from post-ovulation
290 inseminations. In fact, estrogens and progesterone have been shown to influence the
291 time taken for the rat embryo to pass through the oviduct as a result of altering oviductal
292 smooth muscle contraction patterns. As a result, single injection of estradiol administer
293 to rats reduced the embryo's oviductal descent to 20 h as compared with the 72 to 96 h
294 in controls [34]. Human studies have shown a contrary effect of progesterone on the
295 oviductal smooth muscles contractility [35].
296
297 *4.4. Effect of embryo transfer on embryonic growth*
298 The transferred-embryos group was included in the experimental design to determine
299 whether the process of embryo handling during embryo flushing and transfer into
300 asynchronous recipient would detrimentally affect the post-transfer embryo growth in a
301 commercial embryo transfer setting. The results showed no evidence of any delay in
302 embryonic vesicle diameter when compared with non-transferred embryos (**Fig.1**). It
303 appears that the procedures of flushing, pipetting and rinsing to which the embryo is

304 subjected during the 20 to 30 min that remains *in vitro* have no detrimental effect on
305 subsequent growth.
306 Recent evidence has shown that growth of embryos placed into less advanced uteri is
307 retarded [8]. However this study used 10 day embryos and recipients up to 9 days
308 behind the donor. It appears that asynchrony of 1 to 3 days behind the donor is not
309 sufficient to cause a significant delay in post-transfer growth of transferred embryos.
310
311 In conclusion, pregnancies of mares inseminated post-ovulation with either fresh or
312 frozen semen have smaller embryonic vesicles as detected by ultrasound than mares
313 inseminated before ovulation equivalent to approximately 1 day's growth. In a practical
314 setting, especially in embryo transfer centres, this finding has clinical relevance in such
315 that donor mares inseminated post-ovulation should be flushed no earlier than 7.5 to 8
316 days since the interval from insemination to entry of embryo in the uterus in post-
317 ovulation inseminations appears longer than expected as it has been recently suggested
318 [36]. According to data extrapolated from other species, it appears that impaired sperm
319 transport and delayed fertilization after post-ovulation inseminations are the most likely
320 theories to explain this phenomenon. However in the mare it would appear that delayed
321 descent of the embryo is more likely. Serious research studies in the equine species
322 involving different mating protocols and slaughter at different post-insemination times
323 would be needed to elucidate the actual reason for the delay in embryonic growth or
324 transport.
325
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Figure

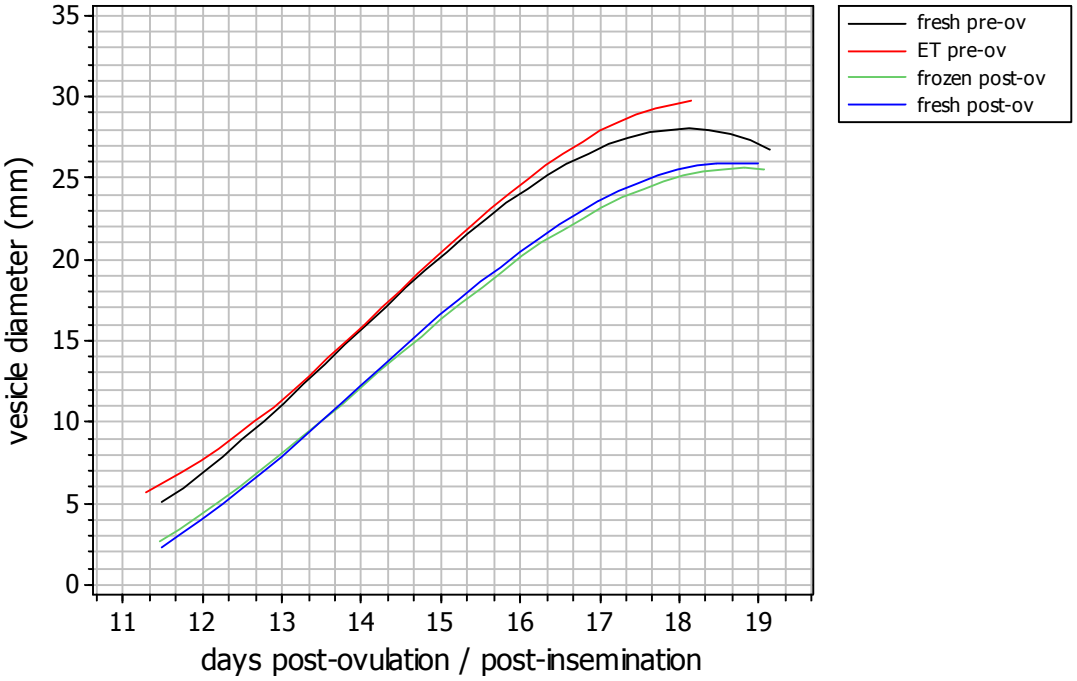

Fig. 1. Quadratic regression fits of vesicle diameters for embryos conceived after pre-ovulation natural mating (fresh pre-ov, n = 42), pre-ovulation natural mating and subsequent embryo transfer to recipient mares (ET pre-ov, n = 39), post-ovulation artificial insemination with frozen/thaw spermatozoa (frozen-post ov, n = 43) and post-ovulation natural mating (fresh post-ov, n = 35).

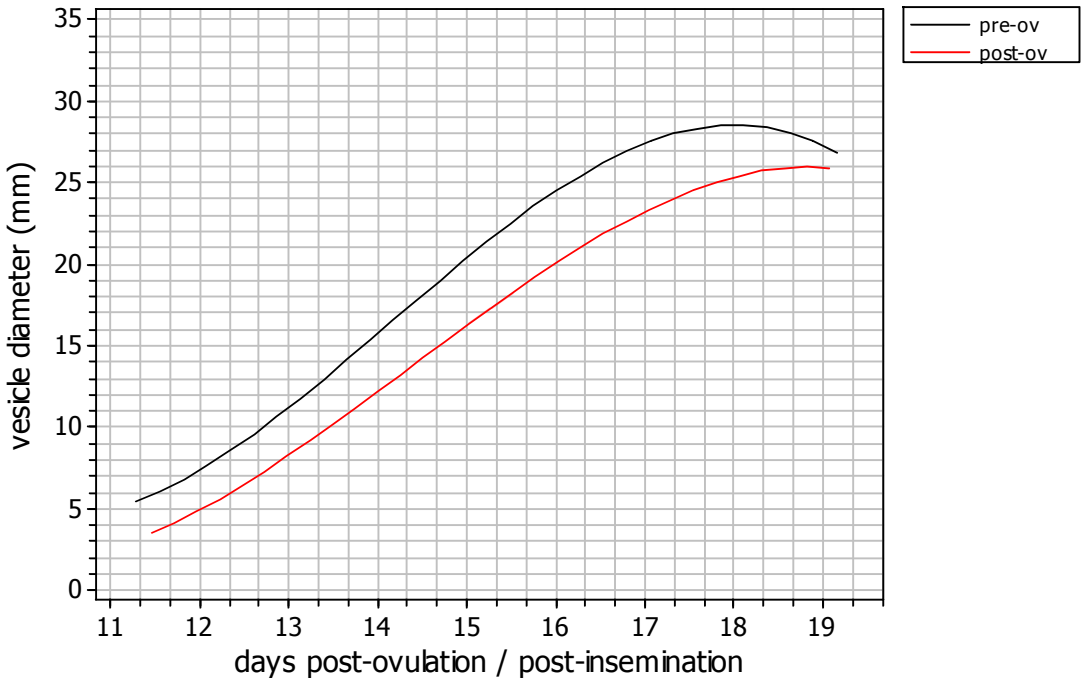

Fig.2. Quadratic regression fits of embryonic vesicle diameters of mares mated naturally pre-ovulation (n = 81 mares) and mares mated post-ovulation with fresh or frozen semen (n = 78 mares).

Tables

Table 1
Mean vesicle diameter ± StDev of embryonic vesicle diameters from different mating protocols

Hours post-ovulation	300	316	324	336	360	372	384	Overall difference (mm)
Fresh-pre (n)	8.9 ± 2.7 (8)	12.4±1.8 (6)	13.9±2.4 (6)	15.8±4.0 (8)	20.1±3.0 (6)	21.5±2.6 (6)	23.8±3.1 (7)	0.1 ± 0.3
ET-pre (n)	9.3 ±2.0 (9)	12.7±3.0 (5)	13.5±2.6 (14)	15.9±2.9 (7)	19.8±2.9 (7)	21.9±2.3 (6)	24.2±2.9 (8)	
P-value	> 0.1	> 0.1	> 0.1	> 0.1	> 0.1	> 0.05	> 0.1	0.73
Hours post-insemination	300	316	324	336	360	372	384	Overall difference (mm)
Fresh-post (n)	5.9±1.6 (6)	9.1±0.8 (6)	10.1±2.6 (6)	11.9±2.9 (11)	15.7±2.2 (5)	17.5±3.5 (2)	19.9±2.4 (6)	0.1 ± 0.4
Frozen-post (n)	6.4±2.3 (6)	9.3±2.4 (7)	10.2±2.8 (6)	12.0±1.6 (6)	16.0±2.9 (8)	19 (1)	19.2±1.6 (6)	
P-value	> 0.1	> 0.1	> 0.1	> 0.1	> 0.1	-	> 0.1	0.71

Fresh-pre: embryonic vesicle diameter (mm) from pre-ovulation natural mating; ET-pre: from pre-ovulation natural mating and transferred into recipient mares; Fresh-post: from post-ovulation natural mating; and Frozen-post: from post-ovulation artificial insemination with frozen/thaw spermatozoa; n: number of mares for each group and time period.

Table 2
Mean vesicle diameter ± StDev of embryonic vesicle from mares mated pre- and post-ovulation

Hours post-ovulation/ insemiantion	300	316	324	336	360	372	384	Overall difference (mm)
Pre-ov (n)	9.1±2.1 (17)	12.6±2.4 (11)	13.5±2.5 (20)	15.8±3.4 (15)	20.0±2.9 (13)	21.6±2.3 (12)	24.0±2.7 (15)	3.7 ± 0.7
Post-ov (n)	6.2±2.0 (12)	9.2±2.7 (13)	10.1±2.6 (12)	11.9±2.6 (17)	15.9±2.6 (13)	18.0±2.7 (3)	19.5±1.4 (12)	
P-value	< 0.01	< 0.05	< 0.05	< 0.01	< 0.01	-	< 0.01	< 0.000

Pre-ov: embryonic vesicle diameter of mares mated pre-ovulation with fresh semen (both transferred and non-transferred embryos); Post-ov: vesicle diameters from mares mated post ovulation (both with fresh and frozen semen); n: number of mares for each group and time period.

Table 3
Mean ± StDev embryonic vesicle growth rate for days 12 to 17 of pregnancy from mares mated pre- and post-ovulation

Day of pregnancy	12	13	14	15	16	17
Pre-ov daily growth rate (n)	3.9 ± 0.9 (7)	4.4 ± 1.3 (10)	4.5 ± 1.1 (7)	3.9 ± 1.3 (9)	3.0 ± 0.9 (4)	1.2 ± 0.9 (3)
Post-ov daily growth rate (n)	2.9 ± 0.5 (7)	4.1 ± 1.0 (12)	4.4 ± 1.1 (9)	4.6 ± 1.8 (7)	3.7 ± 1.8 (3)	2.5 ± 1.1 (3)
P-value	< 0.05	> 0.1	> 0.1	> 0.05	-	-

Pre-ov: embryonic vesicle diameter of mares mated pre-ovulation with fresh semen (transferred and non-transferred embryos); Post-ov: vesicle diameters from mares mated post ovulation (with fresh and frozen semen); n: number of mares for each group

Author Agreement

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