Pferdeheilkunde 20 (2004) 2 (März/April) 145-152

The influence of breed, breeding management and veterinarian on the timing of ovulation and fertility in mares treated with a GnRH analogue, deslorelin (Ovuplant™)

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Summary

During the 1995 equine breeding season, 14 equine practitioners participated in a field trial to evaluate factors influencing the response to treatment with the short acting implant (Ovuplant™) of the GnRH analogue deslorelin, for induction and acceleration of ovulation. Evaluation of 148 individual reports included determination of ovulation (OR), ovulations occurring within 48 hours after treatment (OV-48), the interval between treatment and ovulation (ITO) and the pregnancy rate (PR). Mare factors evaluated included breed, age, parity, reproductive status and mare management. Other factors examined were month of breeding, method of breeding and sperm characteristics, and the practitioner's contribution considering the share of equine clients in the entire patient base, years in practice, and reliability and precision in reporting. Neither breed, nor age nor parity had any influence on the effects of Ovuplant. Maiden mares had the longest ITO (51.9 ±19.5 h) and lactating mares the shortest (40.0 ±11.0 h), with a mean of 44.5 h for all mares. Mare management (breeding of single mares, of mares in stud farms or at breeding stations) had no influence on the treatment effect but mares bred at those breeding stations had low PR rates of 46.9% vs. 57.1 (single mares) and 68.5% (mares at stud farms), respectively. Month of breeding (January to October) had no significant effect on the response to treatment. Insemination with fresh semen resulted in the lowest PR (51.5%), with frozen/thawed semen and natural breeding the PR was 65.0% and 64.7%, respectively, and the PR overall was 57.5%. Differences between veterinarians were highly significant for ITO, approached significance for OV-48 and was again significant for OR with treatments of mares with follicles <30 mm and above. Number of mares treated per participating practitioner had no effects, but the share of horses in the entire client base significantly influences OV-48 for those with only 30% equine clients. Simultaneously, ITO was higher and PR lower. ITO was also influenced by years in practice, with highest values for those longest in practice. Response rates regarding OV-48 and ITO varied significantly between practitioners, and reliability regarding ITO as well.

Key words: mare, ovulation induction, deslorelin, breeding management, veterinarian

Einfluss von Stute, Zuchtmanagement und Tierarzt auf die Ovulationsinduktion und Fruchtbarkeit von mit dem GnRH-Analog Deslorelinazetat (Ovuplant™) behandelten Stuten.

Während der Decksaison 1995 nahmen 14 Tierärzte an einem Feldversuch teil, um mögliche Einflussfaktoren auf die Wirksamkeit des als Kurzzeitimplantat (Ovuplant™) zur Verfügung stehenden GnRH-Analogs Deslorelinazetat zu ermitteln. Die Auswertung von 148 Einzelprotokollen umfasste die Bestimmung der Ovulationsrate (OR), des Auftretens von Ovulationen innerhalb von 48 Stunden nach Deslorelinverabreichung (OV-48), des Intervalls zwischen Verabreichung und Ovulation (ITO) und der Trächtigkeitsrate (PR). Die untersuchten Stutenfaktoren waren Rasse, Alter, Anzahl von Trächtigkeiten, Reproduktionsstatus und Stutenmanagement. Das Besamungs- bzw. Deckgeschehen wurde anhand des Monats der Deckung/Besamung, der Samenart und der Anzahl der Besamungen pro Rosse charakterisiert. Der Beitrag der Tierärzte wurde anhand des Anteils an Pferdebesitzern am Gesamtklientel, der Dauer ihrer Berufsausübung sowie der Glaubwürdigkeit und der Genauigkeit ihrer Berichte beurteilt. Weder Rasse noch Alter und Anzahl der Trächtigkeiten hatten einen Einfluss auf die Wirksamkeit von Ovuplant. Maidenstuten wiesen ein signifikant längeres ITO (51,9 ±19,5 h) im Vergleich zu laktierenden Stuten (40,0 ±11,0 h) auf (P<0,05). Der Durchschnitt aller Stuten betrug 44,5 Stunden. Die Aufstallung der Tiere (Einzeltiere, Gestüte, Deckstationen) hatte ebenfalls keinen Einfluss auf den Erfolg der Ovulationsinduktion; aber die in Deckstationen besamten Stuten wiesen niedrigere PR (46,9 %) gegenüber in Einzelhaltung (57,1 %) oder in Gestüten (68,5 %) gehaltenen Tieren auf. Der Applikationsmonat (Januar bis Oktober) hatte keine Auswirkung auf die Reaktion der Stuten auf die Therapie. Die Besamung mit Frischsamen resultierte in der niedrigsten PR (51,5 %) im Vergleich mit Tiefgefriersamen (65,0 %) und der Deckung (64,7 %). Der Gesamtdurchschnitt der PR lag bei 57,5 %. Die von den Tierärzten erzielten ITO sowie OR unterschieden sich signifikant, wenn Deslorelinazetat bei Stuten mit einem Follikel <30 mm eingesetzt wurde; hinsichtlich der OV-48 wurde das Signifikanzniveau nur knapp verfehlt. Die Zahl der von den einzelnen Praktikern behandelten Stuten hatte keinen Effekt, aber hinsichtlich des Pferdeanteils am Gesamtklientel ergaben sich signifikant niedrigere OV-48 bei den Tierärzten mit 30 %. Desgleichen erreichten das ITO höhere bzw. die PR niedrigere Werte in dieser Gruppe. Das ITO variierte mit der Dauer der Berufsausübung; mit den höchsten Werten für die am längsten praktizierenden Tierärzten. Die Antwortraten der einzelnen Praktiker unterschied sich signifikant bezüglich des OV-48 und des ITO, wie auch die Verlässlichkeit hinsichtlich des ITO variierte.

Schlüsselwörter: Stute, Ovulationsinduktion, Deslorelinazetat, Zuchtmanagement, Tierarzt

Introduction

In modern equine industry, artificial insemination (AI) is used increasingly. As a consequence, breeding management has had to be changed requiring adjustments by all parties involved. Typically, mares display a prolonged estrus in which prediction of the exact timing of ovulation(s) is difficult. Yet in order to inseminate mares close to ovulation, numerous exa-

minations are required, especially in the use of frozen/thawed semen. Hormonal induction of ovulation reduces the number of examinations needed, making breeding management less expensive and conserves stallion power (*Jöchle* et al. 1997), irrespective whether AI is with raw, chilled or frozen semen, or by natural mating.

For hormonal induction and acceleration of ovulation in the mare, the two products most widely used are hCG (human chorionic gonadotropin) and the GnRH analog deslorelin (Ovuplant™). Results obtained with Ovuplant in cyclic mares and their characteristics have been reviewed extensively (*McKinnon* et al. 1993; *Meinert* et al. 1993; *Squires* et al. 1994; *Jöchle* and *Trigg* 1994; *Mumford* et al. 1995; *Meyers* et al. 1997). Deslorelin has also been used subsequent to the administration of progesterone by an intravaginal device (CIDR-B) for the treatment of anoestrous mares during the season (*Arbeiter* et al. 1994) and for the induction of follicle growth and ovulation in transition phase mares (*Arbeiter* et al. 1994; *Handler* et al. 1999; *Newcombe* et al. 2002). This sequence of treatments was also used for the control of cyclic functions in the mare, i.e. the synchronization of estrus (*Lübbecke* et al. 1994) and, in combination with oestradiol, of estrus and ovulation (*Klug* and *Jöchle* 2001).

Own observations during field trials showed that the successful use of Ovuplant and the rate of pregnancies varied greatly between equine practitioners (*Handler* et al. 1995; *Handler* and *Arbeiter* 1996). In addition, differing forms of brood mare management seem have an influence on the success of treatment. Yet mare numbers were too small within each of these practices to allow for a statistical data analysis. Hence, the purpose of the field trials reported here was to evaluate the effectiveness of the deslorelin treatment to induce and time ovulations and to ensure acceptable pregnancy rates after insemination, as influenced by practitioner, mare management, breed of mare and AI procedures.

Materials and Methods

During the 1995 breeding season, 14 participating equine practitioners in Austria (12 M, 2 F) received a total of 245 deslorelin implants (Ovuplant™), a study protocol and individual case report forms. One hundred forty and eight (148, 60.4 %) forms were returned and evaluated.

The protocol stipulated that only mares in clinical estrus were admitted. Mares had to exhibit endometrial oedema and at the time of treatment, a lead follicle 35 to 40 mm in diameter, as determined by ultrasonography. All mares received Ovuplant™, 2.1 mg deslorelin acetate in a short acting, biocompatible implant, subcutaneously at the neck. After treatment with deslorelin, mares were examined per rectum with ultrasonography after 24 and 36 hours and thereafter in 6-hour intervals until ovulation had occurred. If individual practitioners were unable to adhere to this schedule, modifications were negotiated and all deviations from the protocol were recorded. This allowed determination of the interval between treatment and ovulation, from implantation to midway between pre- and post ovulatory examinations (ITO), the percentage of mares that ovulated in response to treatment (OR) and within 48 hrs after treatment (OV-48). Only mares ovulating within 120 hours after treatment with Ovuplant™ were included in the determination of OR and ITO. In mares with multiple ovulations, only the first ovulation was used for determining ITO. First cycle pregnancy rate (PR) was recorded 14 to 18 days after AI or breeding. The following data for mares were collected: breed, age, parity and reproductive status at breeding; the month of treatment, mare management (stud farm; breeding station; mares kept singly) and breeding management, i.e. semen characteristics, exact time of AI or of natural breeding. These factors were used to calculate influences on reproductive success.

In order to evaluate the practitioner's contributions, the following data were requested: The share of equine work in relation to the total client base and the number of years in brood mare practice. The reliability of the individual participant was determined by percentage of returned case report forms, the precision of the information and data provided in each case, the diameter of the lead follicle at treatment and the actual intervals of follow-up examinations until ovulation had occurred.

Tests of the Stat View 4.2 Program (Abacus Concepts, Berkeley, CA, USA) such as ANOVA (parametric), for determining the significance of effects in continuous (ITO) caused by nominal variables, and contingency tables, for evaluating the effects of nominal on dependent nominal variables, were used for descriptive and comparative statistical evaluation. Values were considered to be significant at $P<0.05$.

Results

Of the 148 case report forms received, 91.9% (n=136) contained information about ovulations that occurred in response to treatment and in 91.2% (135 cases) time of ovulation had been determined. A total of 97.8% of all treated mares ovulated (133) and of those, 82.2% (111) within 48 hours, with a mean interval to treatment of 44.9 ±15.4 hours and a range from 16 to 120 hours. Pregnancy examination results were recorded in 138 case reports (93.2%). The overall pregnancy rate was 58% (80/138).

Mare factors

Neither breed, nor age, nor parity had an influence on the effect of Ovuplant™ when examining OR, OV-48, ITO and PR. Reproductive status had a significant effect on ITO ($P<0.05$): in maiden mares (n=31) the mean of 51.9 ±19.5 was longer compared with barren mares (n=36) and open mares, which had not been bred in the last season (n=9), with ITOs of 45.6 ±17.9 and 44.7 ± 4.4 hours, respectively. Nursing mares (n=52) ovulated within 40.9±11.0 hours after deslorelin treatment. Neither OR, nor OV-48, nor PR were influenced by the reproductive status (Table 1).

As shown in Table 2, mare management, like housing mares single (n=28) or at a stud farm (n=54), or temporarily at a breeding station (n=54) had no influence on the induction of ovulation: OR, OV-48 and ITO were not significantly different ($P>0.05$). But the pregnancy rate varied, albeit its significance was questionable ($P=0.0853$). The PR of 46.9% was lower

Tab 1 Effectiveness of Ovuplant™ as determined by ovulation rate (OR), rate of ovulations occurring within 48 hours after treatment (OV-48), the interval between treatment and ovulation (ITO) and the pregnancy rate (PR), as influenced by the mare's breed, age, parity and reproductive status.[1] Includes 4 barren mares, which had been bred unsuccessfully for several years. [2] No data available. [3] Mares not bred during the previous season. * Value differs significantly from non-marked ones (P-values are listed in table). ns – no significant differences

*Effektivität von Ovuplant™ in Abhängigkeit von Rasse, Alter, Anzahl an Trächtigkeiten und Reproduktionsstatus, gemessen an der Ovulationsrate (OR), Rate der Ovulationen innerhalb von 48 h nach der Behandlung (OV-48), Intervall zwischen Behandlung und Ovulation (ITO) und der Trächtigkeitsrate (PR). [1] Enthält 4 Güsstuten, die über mehrere Jahre erfolglos belegt wurden. [2] Keine Daten verfügbar. [3] Stuten wurden in der vorangegangenen Saison nicht belegt. * Wert unterscheidet sich signifikant von unmarkierten (P-Werte sind in der Tabelle angegeben). ns - keine signifikanten Unterschiede*

	n	OR (%)	OV-48 (%)	ITO (h)	PR (%)
Total	148	97,8	82,2	44,9±15,4	58,0
Breed					
Arabs	26	96,2	84,6	41,5±17,7	73,1
Thoroughbreds	1	100	100	42,0	100
Friesians	3	100	100	48,0	33,3
Haflinger	9	100	88,9	48,6±12,1	66,7
Noriker	4	100	100	37,5±3,0	66,7
Standardbred	9	88,9	77,8	44,9±31,3	75,0
Warmblooded horses	84	98,8	79,5	45,8±13,5	49,4
	136	ns	ns	ns	ns
Age (years)					
5 or younger	45	100	82,2	46,4±15,8	55,6
6 to 10	52	96,2	82,4	43,6±14,7	55,6
11 to 15	26	96,2	76,9	45,8±19,5	61,5
16 to 20	4	100	75,0	43,8±6,0	25,0
21 to 25	1	100	100	48,0	100
	128	ns	ns	ns	ns
Parity					
0	35[1]	100	82,9	50,1±19,3	54,1
1	16	93,8	86,7	42,3±13,6	61,1
2	18	100	83,3	46,9±16,7	31,6
3	10	100	70,0	46,7±10,1	63,6
4	4	100	75,0	57,0±26,6	75,0
5	1	100	100	36,0	100
6	3	66,7	33,3	48,0±8,5	50,0
7	1	100	100	36,0	-[2]
10	1	100	100	36,0	-[2]
16	1	100	100	48,0	100
	90	ns	ns	ns	ns
Reproductive Status					
Maiden mares	31	100	80,6	51,9±19,5*	51,5
Nursing mares	52	98,1	84,6	40,9±11,0	58,8
Barren mares	36	94,4	74,3	45,6±17,9	52,8
Open mares[3]	9	100	88,9	44,7±4,4	70,0
	128	ns	ns	P = 0,0211	ns

for mares bred at breeding stations in comparison with the PR of 57.1% and 68.5% for mares kept singly or at stud farms, respectively. Most mares were bred from March to June (n=119), while 16 mares were bred in either, January and February or July to October. Time of the year of treatment with deslorelin had no significant influence on any of the parameters used for measuring treatment success (P>0.05). It had also no effect on PR, although PR appeared to be slightly higher for mares bred in April and May, with 66.7% and 56.8%, respectively. Influences resulting from breeding management are shown in Table 3. Pretreatment of semen (cooling, freezing and thawing), time of breeding in relation to ovulation and number of breedings did not exert any significant effect on the parameters evaluated (P>0.05). The use of diluted fresh semen seemed to reveal lower PR (51.5%) when compared to deep frozen semen and breeding by hand (65.0% and 64.7%, respectively), although the differences were not significant (P>0.05). The PR from breeding prior to ovulation was only 47.1%, as compared with a PR of 63.8% for postovulatory breeding or the PR of 61.5% for the combination of breedings before and after ovulation.

Tab 2 Influence of mare management and the month of treatment on Ovuplant's effectiveness and the pregnancy rate from breeding around the induced ovulations. [1] Includes 4 barren mares, which had been bred unsuccessfully for several years. ns – no significant differences.

Einfluss von Zuchtmanagement und Applikationsmonat auf die Wirksamkeit von Ovuplant und die Trächtigkeitsrate resultierend aus Belegungen zu den induzierten Ovulationen. [1] Enthält 4 Güsstuten, die über mehrere Jahre erfolglos belegt wurden. ns - keine signifikanten Unterschiede.

	n	OR[1] (%)	OV-48 (%)	ITO (h)	PR (%)
Total	148	97,8	82,2	44,9±15,4	58,0
Mare management					
Stud farms	54	98,1	85,2	44,7±15,4	68,5
Breeding stations	54	98,1	77,8	42,9±13,9	46,9
Kept singly	28	96,4	85,2	49,2±18,7	57,1
		ns	ns	ns	ns
Month of treatment					
January	1	100	100	40,0	100
February	6	100	83,3	46,8±4,4	33,3
March	20	100	80,0	47,7±16,7	47,6
April	37	97,3	89,2	43,1±12,4	66,7
May	37	94,6	72,2	47,6±20,8	56,8
June	22	100	86,4	43,3±13,3	47,4
July	6	100	83,3	39,0±14,4	60,0
August	1	100	100	48,0	100
September	1	100	0	48,5	0
October	2	100	100	45,0±4,2	100
		ns	ns	ns	ns

The veterinarians

All 14 practitioners did not differ significantly in the OR and PR they had achieved of 85.7% to 100%, and 0 to 100%, respectively, as shown in Table 4. The wide range of PR among practitioners was due to small mare numbers. While differences for OV-48, ranging from 46.2% to 100%, between practitioners were only marginally significant (P=0.0590), those for ITO were highly significant (P=0.0004). Mean individual ITO values ranged from 24.1±5.8 to 64.5±34.9 hours, yet those of 8 practitioners were seen between 40 and 50 hours post treatment.

Treatments with deslorelin of mares showing follicles of <30 mm in diameter reduced OR significantly to 60% (3/5), while with larger follicles the OR reached 99.2% (128/129), respectively (P<0.0001) (Table 5). Follicle diameter at treatment was without influence on OV-48, 1TO and PR, respectively (Tables 4 and 5). As shown in Table 4, the numbers of treated mares varied greatly between veterinarians, ranging from 2 to 31 mares, respectively, but was without significant

Tab 3 Influence of breeding management, i.e. insemination with fresh or frozen semen, timing of inseminations or of natural service, and number of breedings per oestrus on pregnancy rate (PR). ns – no significant differences

Einfluss des Zuchtmanagements, z.B. Besamung mit Kühl- oder Tiefgefriersamen, Zeitpunkt der Belegungen und die Anzahl der Belegungen pro Rosse auf die Trächtigkeitsrate (PR). ns - keine signifikanten Unterschiede

Breeding management	n	PR (%)
Total	148	58,0
Semen characteristics		
Natural cover	20	65,0
Fresh semen	66	51,5
Frozen semen	51	64,7
Native semen	1	0
	ns	ns
Timing of service		
Preovulatory	51	47,1
Postovulatory	58	63,8
pre- and postovulatory	26	61,5
	ns	ns
Number of breedings		
1	97	60,8
2	34	47,1
3	4	50,0
	ns	ns

	Equine Practitioners															Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
Number of mares treated	2	4	4	4	4	7	7	8	8	13	16	19	21	31		148
Years in equine practice	8	30	12	14	4	23	9	14	3	20	4	14	5	6		12±8
Share of horses in the client base(%)	5,0	5,0	20,0	80,0	5,0	30,0	10,0	40,0	40,0	30,0	20,0	60,0	40,0	20,0		29,0±22,0
Response rate (%)	100	80,0	40,0	20,0	80,0	35,0	100	53,3	100	86,7	53,3	95,0	42,0	100		70,4±28,6
Reliability (%)	81,7	70,8	91,7	81,7	79,2	76,7	92,3	83,8	90,8	91,5	100	90,9	76,0	74,4		84,4±8,5
Lead follicle mean diameter (mm)	34	39	52	39	39	32	34	40	35	40	40	43	42	43		39±5
OR[1] (%)	100	100	100	100	100	85,7	85,7	87,5	100	100	100	100	100	100	ns	97,8
OV-48 (%)	100	100	100	100	50,0	66,7	85,7	87,5	75,0	46,2	93,8	89,5	80,9	89,5	ns	83,2
ITO (h)	48,0	37,5*	44,8	34,5*	55,2*	64,5*	36,3*	24,1*	49,4	47,6	44,0	40,0	48,6	48,2	P=0.0004	44,5
PR (%)	50,0	0	100	-[2]	75,0	60,0	66,7	75,0	50,0	38,5	62,5	47,4	66,7	54,8	ns	57,5

Tab 4 Comparison of treatment responses to Ovuplant and the pregnancy rate from breeding around the induced ovulation by 14 equine practitioners. [1] Includes 4 barren mares, which had been bred unsuccessfully for several years. [2] No data available. * Value differs significantly from non-marked ones (P-values are listed in table). ns - no significant differences.
*Vergleich zwischen 14 Tierärzten hinsichtlich des Behandlungserfolges mit Ovuplant und der erzielten Trächtigkeitsraten von Belegungen während der induzierten Rossen. [1] Enthält 4 Güsstuten, die über mehrere Jahre erfolglos belegt wurden. [2] Keine Daten verfügbar. * Wert unterscheidet sich signifikant von unmarkierten (P-Werte sind in der Tabelle angegeben). ns - keine signifikanten Unterschiede.*

effect on all four parameters (OR, OV-48, ITO and PR) (P>0.05).

The number of horses in relation to the entire number of clients serviced by the participating practitioners ranged from 5% to 80% (Table 4). This parameter had no significant effect on the OR, on ITO and PR (P>0.05), but was highly significant regarding OV-48 (P=0.0151), mainly due to two veterinarians with a 30% equine clientele (Table 4). Taken together, their OV-48 value of 52.6% was clearly below those values for all others, which ranged from 80 % to 100%. Those two practitioners reported also higher values for ITO (52.9 hours vs. 34.5 to 46.7 hours) and a lower PR (44.4% vs. 47.4 to 66.7%).

The number of years participants had worked in equine practice influenced ITO significantly (P=0.0056). Participants with 21 to 30 years in practice reported a higher ITO of 53.7±29.5 hours versus practitioners with <10 years or 10 to 20 years of equine practice, with ITOs of 47.0±12.2 and 39.7±14.7 hours, respectively. The same factors did not cause significant differences for the parameters OR, OV-48 and PR (P>0.05).
As shown in Table 4, the rate of response varied greatly between participants and ranged from 20% to 100%. Significant differences were found for OV-48 (P=0.0289) and ITO (P=0.0067), but there was no correlation with the rate of returned case reports. The reliability of the individual participants was determined by calculating the percentage of entries into case report forms. Those entries dealt with the effectiveness of the treatment with deslorelin and the level of fertility observed. They ranged from 70.8% to 100%. ITO values in the group of practitioners which provided 81-90% of all entries was significantly lower (P=0.0001) when compared with the lower ranking (70-80%) and the higher ranking groups (91-100%) of 31.0±10.5 hours vs. 49.9±17.5 and 43.5±12.3 hours, respectively (Table 4).

Tab 5 Influence of follicle diameter at implantation on Ovuplant's effectiveness. [1] Includes 4 barren mares, which had been bred unsuccessfully for several years. * Value differs significantly from non-marked ones (P-values are listed in table). ns – no significant differences
*Einfluss des Follikeldurchmessers bei Implantation von Ovuplant auf seine Wirksamkeit. [1] Enthält 4 Güsstuten, die über mehrere Jahre erfolglos belegt wurden. * Wert unterscheidet sich signifikant von unmarkierten (P-Werte sind in der Tabelle angegeben). ns - keine signifikanten Unterschiede*

	n	OR[1] (%)	OV-48 (%)	ITO (h)
Follicle diameter (mm)				
25-30	5	60.0*	75.0	48.0
31-35	29	100.0	93.1	42.3±9.7
36-40	45	97.8	75.5	43.9±10.5
41-45	33	100.0	81.8	46.2±18.2
46-50	18	100.0	88.9	42.5±16.3
> 50	4	100.0	100.0	41.3±4.6
	134	P < 0.0001	ns	ns

Discussion

Despite of the differing conditions of use, deslorelin reliably accelerated and timed ovulation of mares in oestrus. The overall response rate of 87.8% and the rate of 82.2% of these mares ovulating within 48 hours confirmed similar values reported previously by *Handler* and *Arbeiter* (1996), *Jöchle* and *Trigg* (1994) and *Meyers* et al. (1997). The mean interval between treatments and ovulations (ITO) of 44.9 h corresponded well with the range of intervals given with 40.5 and 49.1 h in prior study reports by *Handler* and *Arbeiter* (1996), *Klug* et al. (1992), *McKinnon* et al. (1993 and 1997), *Meinert* et al. (1993) and *Meyers* et al. (1997). Of the factors evaluated, only the mares' reproductive status had a significant effect on the product effectiveness, while age, breed and parity had no effect. The interval from treatment to ovulation was longest in maiden mares (51.9 h) and shortest (40.9 h) in mares nursing a foal. This contrasts with observation on lactating mares previously reported by others (*Jöchle* and *Trigg* 1994; *Meyers* et al. 1997). In a preliminary report on part of the data shown here we had described differences between breeds in the response to treatment (*Handler* and *Arbeiter* 1996). With larger numbers of mares evaluated, such differences were no longer observed.

Mare management and month of treatment had no influence on the treatment's effectiveness. Hence, neither environmental factors like housing mares singly, or at stud farms or bree-

ding station where there was contact with other horses, or being teased by stallions, nor seasonal factors exerted significant effects on the response to treatment with deslorelin. Our results differ from those reported by *Farquhar* et al (2000), based on a total of 376 Ovuplant treated mares from 1995 to 1999. These authors observed an overall OV-48 rate of 84.3% and an OV-72 of 90.9%. The OV-48 for 10 to 14-year old mares was significantly higher and that of mares 20 years and older. In their population, a significantly higher OR and shorter ITO were seen for treatments during July to October and the lowest values with treatments in March and April. Larger numbers of treated animals and differences in breed, management, latitude and altitude of one versus 14 different locations may account for these differences, although the overall values reported in their study are very similar to ours.

As had been shown in a preliminary report with a smaller number of cases (*Handler* and *Arbeiter* 1996), individual practitioners exerted a significant effect on the response to treatment, especially on the interval treatment to ovulation (ITO). While the number of mares treated per practitioner was without effect on ITO, significant interactions were seen with the following factors: percentage of horses in the client base; rate of response, i.e. return of reports; reliability in answering all questions raised in the report form and years in practice. The impression was gained that the first two of these factors also had influenced the values for OV-48 (n.s.). But it was not possible to establish linear relationships and/or any correlation between ITO and these or other factors. A strong relationship was observed between lead follicle diameters at treatment and its effects: lead follicles <30 mm in diameter had an OR of only 60% versus an OR of close to 100% when mares with larger follicles were treated. This observation is in agreement with data reported by *Jöchle* and *Trigg* (1994) and might reflect the inability of an immature follicle to respond to an ovulation-inducing boost of LH. This underlines the need for ultrasonography as an indispensable tool for determining the correct time for breeding, especially AI, and for treatment with deslorelin.

In 60% of the mares, which were treated with a lead follicle of <30mm yet responded with ovulation, OV-48 and ITO did not differ from values in mares treated when lead follicles were larger. How follicle diameter at treatment can influence the treatment's effectiveness is shown when comparing the data from practitioners 6 (P6) and 8 (P8) in Table 4. The clientele of P6, mostly large-framed, warm blooded sports horses, were treated when follicle diameters were between 28 and 36 mm. P8 treated Arabian mares only when follicle diameters were approaching 40 mm. As a consequence, the ITO for the former was extended to 64.5 h and shortened for the latter to 24.1 h, indicating that P6 treated his or her population too early and P8 almost too late. *Jöchle* and *Trigg* (1994) had already pointed to breed-related differences in the size of preovulatory follicles and the need to adjust treatment criteria accordingly. With P10 another factor was identified as the cause of deviating results, i.e. low values for OV-48 and PR. This practitioner was unable to adhere to the 6-hour intervals in post treatment examinations, which were actually performed in intervals of 12 to 19 h. Hence, ovulations in some cases had been detected late and post ovulatory AI may have been too late (Table 4).

With 58.0%, the pregnancy rate per cycle overall after treatment with deslorelin was in agreement with rates given by many sources. Mare related factors like breed, age, parity and reproductive status had no significant effect on the PR after this hormonal induction of ovulation. Even barren mares, with a PR above 50%, responded better than anticipated. While the PR did not show seasonal variation, brood mare management did: mares treated and bred at breeding stations (stallion stations) showed a lower (albeit barely significant) PR than mares at stud or kept singly. Factors, which may have contributed to this reduction, were the transport of mares to these stations when first signs of heat were observed, the new environment to which these mares were exposed, and the high percentage (58.5%) of inseminations with fresh semen. Overall, in this study, the PR from AI with frozen/thawed semen and natural cover were 64.7% and 65.0%, respectively, while the PR with fresh semen was 51.5%. These differences were not significant.

Fresh semen was used frequently prior to ovulation. As shown in Table 3, preovulatory insemination yielded a lower PR than postovulatory AI or double AI before and after ovulation (n.s.). *Handler* and *Arbeiter* (1997) had shown that in 97 mares, with AI before and after ovulation, semen characteristics (fresh vs. chilled vs. frozen) and the frequency of AI, had no significant effect on PR: all PRs were within the range of values of 49.7% for fresh semen and 55.6% for two inseminations or breedings by hand. Only single breedings by hand achieved a PR of 77.8%. Other authors have reported differing PRs and/or foaling rates when using differently prepared semen for AI or natural cover (*Pace* and *Sullivan* 1975; *Klug* 1992) or inseminating at different times in relation to ovulation (*Woods* et al. 1990). In addition, PRs in barren mares often are significantly lower (*Baker* et al. 1993; *Barbacini* et al. 1999a, 1999b). But it is often difficult to compare data on fertility from published reports by others since reproductive success is expressed by a variety of differing parameters, like PR per cycle, per breeding season or based on the foaling rate.

Conclusions and Implications

This field trial once more confirmed that in the mare ovulation can be reliably induced and accelerated with the GnRH analog deslorelin (Ovuplant ™) and that the pregnancy rate, irrespective of the method of breeding, is very satisfactory. Evaluations of potentially influential factors, including the mare's reproductive history, status and management, the method of breeding and the equine practitioner's actions revealed only minor impacts or none. The exception was significant differences in the results achieved between the participating equine practitioners. In the final analysis these were caused by non-adherence to guidelines for treatment and evaluation. Our results should encourage to address these factors in the instructions for use to veterinarians and to educate practitioners continuously about the possibilities and limitations of such biotechnical procedures.

Ovuplant™ is an example for non-therapeutic drugs, which may either enhance performance or the predictability of reproductive processes. Their use often requires an exact timing of follow-up procedures, like determining in this case

that ovulations are imminent or have already occurred, and the scheduling of inseminations or breedings. The law restricts the use of such drugs to veterinarians. As this study shows, not all veterinarians have grasped these consequences fully.

Acknowledgement

The authors would like to thank Dr. T. E. Trigg, Peptech Animal Health, North Ryde, NSW Australia, for making Ovuplant™ available, and John R. Newcombe, Brownhills, West Midlands, for valuable input.

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