Effect of exogenous progesterone on superovulatory response in heifers inseminated with fresh or frozen semen

D. Goulding, D. H. Williams, J. F. Roche and M. P. Boland*

National Agricultural and Veterinary Biotechnology Programme, University College Dublin,
Belfield, Ireland

Superovulation in cattle normally involves the administration of gonadotrophins at specific times of the oestrous cycle, followed by the induction of luteolysis and insemination with high quality semen. The first aim of this experiment was to examine the effect of supplementary progesterone when used in conjunction with porcine FSH (pFSH) to induce superovulation in heifers. The methods compared were PGF_{2α} given at mid-cycle or a progesterone-releasing intravaginal device (PRID) inserted at different phases of the cycle. The second aim was to determine whether site of insemination or use of fresh or frozen semen affected embryo production. A factorial design was used involving 185 beef heifers. The main factors were (i) synchronization methods (PGF_{2α} or PRID); (ii) semen type (fresh or frozen); (iii) insemination regimens (involving two inseminations and variations in the sites) and number of straws used (one or two) at the second insemination. Eight injections of pFSH were given twice a day for 4 days starting either on days 9, 10 or 11 of the oestrous cycle or on the fourth day after insertion of a PRID. Heifers were checked for oestrus, inseminated twice and embryos were recovered on day 7 of the superovulated cycle. There was no difference between heifers given either PRID or PGF_{2α} in the oestrous response (93% versus 96%), number of ovulations (15.9 ± 1.11 versus 13.4 ± 1.06), large follicles (2.5 ± 0.24 versus 2.3 ± 0.23) or embryos recovered (9.1 ± 0.77 versus 9.1 ± 0.74). The number of embryos that could be frozen was lower ($P = 0.05$) in heifers given PRID. The stage of the cycle at which the PRID was inserted affected the number of ovulations, large follicles and embryos recovered ($P < 0.04$). The use of fresh or frozen semen had no effect on the number of embryos recovered, but the use of frozen semen resulted in fewer grade 1 and 2 embryos and more grade 4 and 5 embryos in PRID-treated heifers. The number of straws did not affect the number or quality of embryos recovered. In conclusion, the use of a PRID, inserted at different stages of the cycle, in conjunction with PGF_{2α} and pFSH resulted in fewer freezable embryos recovered compared with the use of PGF_{2α} and pFSH given at mid-cycle. The use of frozen semen did not affect the number or quality of embryos recovered following the use of PGF_{2α} and pFSH at mid-cycle, but it did decrease the number of grade 1 and 2 embryos recovered following the use of PRID and pFSH; the number (two versus three) of straws used did not affect the yield or quality of embryos recovered.

Introduction

The current gonadotrophin of choice for effective superovulation and high embryo recovery rates in cattle is porcine FSH (pFSH) (Boland *et al.*, 1991) because it results in significantly more embryos recovered compared with the use of equine chorionic gonadotrophin. The stage of the oestrous cycle at which the gonadotrophin treatment is initiated affects the ovulatory response, owing presumably to the sequential periods of selection of a dominant follicle and

exertion of the dominance effects throughout the oestrous cycle (Ireland and Roche, 1987; Savio *et al.*, 1988; Sirois and Fortune, 1988). The current most common protocol to superovulate cattle is injections of pFSH twice a day for 4 days between days 9 and 12 of the cycle in association with the use of a luteolytic dose of PGF_{2α} on the third day of treatment (Boland *et al.*, 1991). This method, however, requires selection of animals for treatment based on a specific day of the oestrous cycle. In contrast, ewes can be effectively superovulated using gonadotrophins given towards the end of a 12–14 day progestagen treatment, initiated without reference to the stage of the cycle (Boland *et al.*, 1983). However, there are relatively few comparable data available

*Correspondence and reprint requests.
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on superovulation in conjunction with progestagen treatment in cattle (Holtz *et al.*, 1979; Almeida, 1987). If multiple injections of FSH at, or near the end of, a progesterone-treatment period give results similar to the use of FSH and PGF_{2α} at mid-cycle, the use of progesterone will obviate the need to detect oestrus before initiation of superovulation of donors. Accordingly, the first aim of this experiment was to compare embryo production rates in heifers given pFSH either at a known stage of the cycle with PGF_{2α} or towards the end of a 7 day progesterone treatment, and PGF_{2α} where treatment was initiated at three different stages of the cycle.

The use of progesterone to synchronize oestrus in ewes results in a requirement for high numbers of spermatozoa to achieve normal fertility (Boland *et al.*, 1983). In addition, freezing of spermatozoa increases the number of dead spermatozoa, decreases motility after thawing and reduces their fertilizing capacity. In multiple ovulating animals, such as sows, there is a relationship between the establishment of a viable proportion of highly fertile spermatozoa at the utero-tubal junction and isthmus of the oviduct and fertilization rate (Hunter, 1988). Failure of a proportion of eggs to be fertilized in superovulated cattle may be associated with an initial depletion of the functional sperm reservoirs and a subsequent shortage of viable sperm cells in the proximal portion of the oviduct during the protracted period of ovulation (Hunter, 1988). A means of overcoming the diminished fertility might be to combine the routine technique of intrauterine insemination with simultaneous deposition of spermatozoa in the cervix. A prolonged release and migration of spermatozoa from the cervical mucus could then compensate for a depleted reservoir in the oviduct. Accordingly, the second aim of this experiment was to determine the effect of number of straws used, the use of fresh or frozen-thawed semen and varying the site of semen deposition (cervical or uterine) on embryo production in superovulated heifers.

Materials and Methods

Animals and treatments

A group of 185 Simmental and Charolais cross-heifers aged 18–24 months were maintained in a slatted unit with access to water *ad libitum*. They were fed a complete diet of silage, brewers grains and corn gluten that was calculated to give a minimum daily liveweight gain of 0.7 kg day⁻¹. The reproductive tracts of all heifers were palpated via the rectum before the study to establish normality and to allow selection of non-pregnant heifers. Heifers were given 15 mg of a PGF_{2α} analogue (luprostiol; Prosolvin; Intervet Ltd, Boxmeer) to synchronize oestrus before superovulation. Oestrous detection was carried out for 20 min four times a day at 07:00, 12:00, 17:00 and 21:00 h by visual observation (day of oestrus = day 0).

A factorial design was used and the main factors were: (i) synchronization methods that were either an injection of PGF_{2α} analogue alone (cloprostenol, Estrumate, Coopers, Bray, Co. Wicklow) at mid-cycle (days 11–13; *n* = 91) to induce luteolysis or the insertion of a progesterone-releasing intra-vaginal device (PRID; Sanofi Animal Health, Watford) without

Fig. 1. Superovulation schedule used for heifers. PGF_{2α} was used alone, or in conjunction with a progesterone-releasing device (PRID), to induce luteolysis following injections of porcine FSH given twice a day for 4 days. [†]The PRID was inserted on days 1–3, 6–8 or 16–18 of the oestrous cycle. AI: artificial insemination. ↓ each injection of FSH. * Injections of prostaglandin analogue.

Table 1. Site of insemination and number of straws used for insemination of heifers

Insemination	First	Second	•
Number of straws	1	1	1
Placement of semen	Uterus	Cervix	—
	Uterus	Uterus	—
	Uterus	Uterus	Cervix
	Uterus	Uterus	Uterus

the oestradiol benzoate capsule for 7 days and PGF_{2α} given on the sixth day of PRID treatment, with PRIDs being inserted during the early luteal (days 1–3; *n* = 35), mid-luteal (days 6–8; *n* = 25) or late luteal (days 16–18; *n* = 24) stages of the cycle, respectively (Fig. 1); (ii) use of fresh or frozen-thawed semen; (iii) site of semen deposition (intrauterine or cervical placement) and number of semen straws (two or three) (Table 1).

The pFSH (Folltropin; Vetrepahrm, London, Ontario) was given twice a day for 4 days using doses of 4.5, 3.5, 2.5 and 1.5 mg per injection day⁻¹, respectively, (total dose of 275 mg NIH-FSH-P1 per heifer). The pFSH injections were started on days 9–11 of the oestrous cycle in the heifers given PGF_{2α} and on the fourth day of progesterone treatment (60 h before PRID withdrawal) in the PRID-treated heifers. Luteolysis was induced in all heifers by injections of cloprostenol at the fifth (500 µg) and sixth (250 µg) pFSH injections; the PRID was removed at the time of the second PGF_{2α} injection. Observations for standing oestrus began 24 h after the second PGF_{2α} injection. The first insemination was carried out by the same technician 8–12 h after the onset of oestrus and the second approximately 12 h later.

Semen from two bulls of known high fertility was used throughout the experiment. A single ejaculate of comparable quality was obtained each morning, and the ejaculate was split

Table 2. Superovulatory response (injections of porcine FSH twice a day for 4 days) and embryo production rate (mean $\pm$ SEM) in heifers given PGF_{2 α} at mid-cycle (days 11–13), or a 7-day PRID treatment and PGF_{2 α} before PRID removal. PRIDs were inserted at the early, mid- or late luteal phase of the oestrous cycle

Number of	Method of synchronization	
	PGF _{2α}	PRID plus PGF _{2α}
Heifers treated	95	90
Heifers in oestrus	91	84
Ovulations	13.4 $\pm$ 1.06	15.9 $\pm$ 1.11
Large follicles (> 10 mm)	2.3 $\pm$ 0.23	2.5 $\pm$ 0.24
Embryos recovered	9.1 $\pm$ 0.74	9.1 $\pm$ 0.77
Freezable embryos (grade 1 and 2)	4.4 $\pm$ 0.41 ^a	3.2 $\pm$ 0.43 ^b
Transferable embryos (grade 3)	2.0 $\pm$ 0.26	2.5 $\pm$ 0.27
Degenerate embryos (grades 4 and 5)	1.9 $\pm$ 0.40	2.9 $\pm$ 0.42
Unfertilized ova	0.9 $\pm$ 0.23	0.9 $\pm$ 0.24

PGF_{2 α} : prostaglandin F_{2 α} . PRID: progesterone-releasing intravaginal device. ^{ab}Means within a row with different superscripts differ significantly ($P < 0.05$).

into two aliquots, one was frozen immediately (50×10^6 spermatozoa per straw), and the fresh aliquot was diluted (25×10^6 spermatozoa per straw) in Caprogen diluent, stored at 4°C and used the same afternoon and the following morning.

Hormone determination

Jugular blood samples were collected at the time of the first, third, fifth, sixth, seventh and eighth pFSH injections. Samples were kept at room temperature for 1 h and stored at 4°C overnight. After centrifugation at 700 *g* for 20 min, the serum was decanted and stored at –20°C until assayed for progesterone using the non-extraction assay of Ronayne and Hynes (1990). The inter- and intra-assay coefficients of variation for a pool of samples containing 0.4, 2.2 and 3.5 ng progesterone ml^{–1} were 11.7, 7.8 and 10.9% and 2.4, 1.0 and 2.8%, respectively. The sensitivity of the progesterone assay was 0.1 ng ml^{–1}.

Embryo classification

Heifers were killed seven days after oestrus and the reproductive tracts recovered within 20 min of death. The numbers of corpora lutea and large follicles (≥ 10 mm) on each ovary were recorded. Each reproductive tract was severed at the uterotubal junction and the uterine horns flushed separately with 20 ml of phosphate-buffered saline and 3% heat-inactivated steer serum (Goulding *et al.*, 1990). Embryos were recovered, counted, examined and morphologically graded by the same operator on a scale of 1–5 (Boland *et al.*, 1978). Grade 1 and 2 embryos were classified as suitable for freezing, grade 3 embryos were classified as suitable for transfer only, while grade 4 and 5 embryos were of poor quality or degenerate.

Statistical analysis

The effect of synchronization, semen type and site of semen deposition on variables relating to embryo quality and

recovery were analysed using ANOVA methods appropriate to a three factor completely randomized design. Least square means were used for comparison purposes. The model mean square error term was used to estimate SEMs and linear contrasts. The mean progesterone concentrations at the time of pFSH injections were tested for significant differences using a *t* test. The rates of progesterone decline after cloprostenol administration were compared by regressing the logarithm of progesterone concentrations against time.

Results

Method of synchronization

The method of synchronization did not affect the oestrous response, number of ovulations, large follicles, embryos recovered or transferable and degenerated embryos (Table 2). However, the number of freezable embryos was lower in heifers given pFSH in association with PRID compared with PGF_{2 α} at mid-cycle (Table 2). The stage of the cycle at the time of PRID insertion affected the superovulatory response obtained; the numbers of ovulations, large follicles and embryos recovered were significantly lower ($P < 0.04$) in heifers given PRIDs during the early luteal phase than at other stages of the oestrous cycle (Table 3). Heifers treated during the early luteal phase had significantly fewer ($P = 0.01$) large follicles than did heifers treated during the mid-luteal phase. There were significantly more ($P = 0.03$) transferable embryos recovered from heifers given a PRID during the late luteal phase compared with during the early phase of the cycle.

Type of semen

The use of fresh or frozen–thawed semen had no effect on the number of embryos recovered but the use of frozen–thawed semen in heifers given a PRID resulted in the recovery of fewer grade 1 and 2 embryos and an increase in the number of grade 4 and 5 embryos, compared with heifers receiving

Table 3. Superovulatory response (injections of porcine FSH twice a day for 4 days) and embryo production rate (mean $\pm$ SEM) in heifers given PGF_{2 α} at mid-cycle (days 11–13), or a 7-day PRID treatment and PGF_{2 α} before PRID removal. PRIDs were inserted at the early, mid- or late luteal phase of the oestrous cycle

Number of	Stage of the cycle at PRID insertion			PGF _{2α}
	Early	Mid	Late	
Heifers	35	25	24	91
Ovulations	11.8 $\pm$ 1.66 ^a	18.5 $\pm$ 1.96 ^b	19.1 $\pm$ 2.04 ^b	13.3 $\pm$ 1.03 ^a
Large follicles	1.7 $\pm$ 0.37 ^a	3.2 $\pm$ 0.44 ^b	2.8 $\pm$ 0.45 ^{ab}	2.3 $\pm$ 0.23 ^{ab}
Embryos recovered	6.4 $\pm$ 1.13 ^a	10.5 $\pm$ 1.46 ^b	11.0 $\pm$ 1.47 ^b	9.1 $\pm$ 0.70 ^b
Freezable embryos (grades 1 and 2)	3.0 $\pm$ 0.66 ^a	3.3 $\pm$ 0.79 ^{ab}	3.5 $\pm$ 0.82 ^{ab}	4.4 $\pm$ 0.41 ^b
Transferable embryos (grade 3)	1.6 $\pm$ 0.40 ^a	2.8 $\pm$ 0.48 ^{ab}	3.4 $\pm$ 0.52 ^b	2.0 $\pm$ 0.23 ^a
Degenerate embryos (grades 4 and 5)	1.6 $\pm$ 0.63	3.4 $\pm$ 0.74	3.4 $\pm$ 0.81	1.9 $\pm$ 0.38
Unfertilized ova	0.5 $\pm$ 0.37	1.3 $\pm$ 0.44	1.1 $\pm$ 0.46	0.9 $\pm$ 0.23

PGF_{2 α} : prostaglandin F_{2 α} . PRID: progesterone-releasing intravaginal device. ^{ab}Means within a row with different superscripts differ significantly ($P < 0.05$).

Table 4. Superovulatory response (injections of porcine FSH twice a day for 4 days) and embryo production rate (mean $\pm$ SEM) in heifers given PGF_{2 α} at mid-cycle (days 11–13), or on the fourth day of a 7-day PRID treatment. Heifers were inseminated with fresh or frozen–thawed semen (results pooled across different sites and numbers of straws)

Number of	Method of synchronization			
	PGF _{2α}		PRID	
	Semen type		Semen type	
	Fresh	Frozen	Fresh	Frozen
Heifers	47	48	48	42
Heifers in oestrus	43	48	44	40
Ovulations	14.0 $\pm$ 1.54	12.8 $\pm$ 1.45	15.8 $\pm$ 1.52	15.9 $\pm$ 1.61
Embryos				
recovered	8.9 $\pm$ 1.07	9.3 $\pm$ 1.02	8.6 $\pm$ 1.06	9.7 $\pm$ 1.13
grades 1 and 2	4.3 $\pm$ 0.60 ^a	4.4 $\pm$ 0.56 ^a	3.9 $\pm$ 0.59 ^{ab}	2.5 $\pm$ 0.62 ^b
grade 3	2.0 $\pm$ 0.38	2.1 $\pm$ 0.36	2.2 $\pm$ 0.37	2.9 $\pm$ 0.40
grades 4 and 5	1.9 $\pm$ 0.58 ^a	2.0 $\pm$ 0.55 ^a	2.0 $\pm$ 0.57 ^a	3.8 $\pm$ 0.61 ^b
Unfertilized ova	0.9 $\pm$ 0.33	0.8 $\pm$ 0.32	0.5 $\pm$ 0.33	1.4 $\pm$ 0.35

PGF_{2 α} : prostaglandin F_{2 α} . PRID: progesterone-releasing intravaginal device. ^{ab}Means within a row with different superscripts differ significantly ($P < 0.05$).

PGF_{2 α} and frozen–thawed semen (Table 4). The number of straws of semen used did not affect the number or quality of embryos recovered.

Site of semen deposition

In general, the only effect of site of semen deposition was on the number of grade 3 embryos recovered. The number of grade 3 embryos recovered following uterine deposition of semen was higher than the number recovered after uterine and cervical semen placement (3.1 $\pm$ 0.36 versus 2.0 $\pm$ 0.22; $P = 0.01$).

Progesterone concentrations

Progesterone concentrations in treated heifers are given (Fig. 2). At the time of the first, third and sixth pFSH injections,

progesterone concentrations in heifers treated with a PRID or given PGF_{2 α} in the mid-luteal phase of the cycle were higher than in heifers treated with a PRID during the early luteal phase; mid-luteal phase heifers had higher progesterone concentrations than did late-luteal phase heifers ($P < 0.05$). After PGF_{2 α} injection, the decline in progesterone concentration of heifers given a PRID in the late luteal phase differed from that of heifers given PRID in the early and mid-luteal phase or given PGF_{2 α} alone ($P < 0.03$).

Discussion

The main finding from this experiment was that exogenous progesterone treatment during superovulation may alter the yield of high quality embryos recovered. However, there was no major effect of use of fresh semen, site of insemination or

Fig. 2. The mean ($\pm$ SEM) progesterone concentration at time of porcine FSH injections in superovulated heifers in which luteolysis was induced with prostaglandin F_{2α} (◇) alone or with a progesterone-releasing intravaginal device inserted at (□) early, (◆) mid- or (■) late luteal phase of the oestrous cycle. Arrows indicate prostaglandin F_{2α} injections.

number of straws used on embryo yield. The use of progesterone to synchronize oestrus obviates the need to know the specific stage of the oestrous cycle when starting the superovulatory treatment, but this management benefit must be offset against the decrease in the numbers of freezable embryos recovered. There was a clear effect of stage of the cycle at which the PRID was inserted on the number of ovulations and embryos recovered. In practical terms, progesterone treatment should not always be used in donors, but it can be selectively used in heifers from day 6 onwards or in those not detected in oestrus. This will involve one week of oestrous detection and the exclusion of those heifers detected in oestrus from treatment with progesterone.

The lower ovulatory responses to pFSH following PRID insertion in the early luteal phase suggests that the ovary is less responsive to exogenous gonadotrophins than at the mid- and late luteal phase. This result is consistent with other studies in the early luteal phase (Lindsell *et al.*, 1985; Goulding *et al.*, 1990). Initiation of gonadotrophin treatment in the early luteal phase, which in this study was between days 5 and 7 of the cycle, is coincident with the establishment of the first dominant follicle (Savio *et al.*, 1988; Sirois and Fortune, 1988; Knopf *et al.*, 1989). The suppressive effect of the dominant follicle on other recruited follicles in that cohort possibly contributed to a reduced superovulatory response (Grasso *et al.*, 1989; Guilbault *et al.*, 1991). The lowered response cannot be attributed to lower progesterone concentrations, in the early luteal phase, since they were similar to those during the late luteal phase. It is known that progesterone treatment given from the beginning of the cycle will not alter the response to a single injection of pFSH (Vaughan, 1990). However, it is not clear whether pFSH treatment can rescue small atretic follicles leading to the ovulation of aberrant oocytes.

The lowered ovulatory response of heifers given a PRID during the early luteal phase was reflected in the number of embryos recovered. Even though the number of ovulations did not differ significantly between the early luteal phase PRID-treated heifers and the PGF_{2α}-treated heifers, significantly

fewer embryos were recovered from heifers given PRID in the early luteal phase. This suggests impairment of embryo transport or greater early embryo loss in the PRID-synchronized heifers when treatment was started in the early luteal phase compared with the PGF_{2α}-heifers. It is therefore clear that superovulation treatments should not be initiated early in the luteal phase, irrespective of method of synchronization used (Goulding *et al.*, 1990).

The numbers of ovulations and of large follicles were not different between the PGF_{2α} and PRID treatments when data for all stages of the cycle were combined. This is in agreement with other data reported for use of the PRID, although using a different experimental design (Kunkel, 1979). Other studies have been carried out incorporating the synthetic progestagen, norgestomet, to regulate the cycle in a superovulatory regimen, but the results are equivocal with some authors reporting no effect on the ovulatory response (Holtz *et al.*, 1979; Prather *et al.*, 1984; Hill *et al.*, 1986), whereas others reported a detrimental effect (Almeida, 1987; Garcia-Winder *et al.*, 1988). Information concerning the precise stage of the oestrous cycle at which norgestomet treatment was started might facilitate interpretation of these conflicting results.

In the study reported here the oestradiol capsule was removed from the PRID at insertion and this may have influenced the response. Progesterone concentrations resulting from use of a PRID for 7 days would not affect the turnover of dominant follicles in heifers during the luteal phase, but would delay ovulation by 1–3 days in heifers treated during the follicular phase. It is known that oestradiol can affect luteal function and could also affect the pattern of turnover of dominant follicles during the oestrous cycle of treated heifers. Administration of oestradiol at the time of norgestomet insertion has no effect on the ovulatory response (Wu *et al.*, 1988; Bo *et al.*, 1990, 1991), but results in fewer transferable embryos (Bo *et al.*, 1990). When oestradiol injection was given at the same time as pFSH injection, rather than at norgestomet insertion, superovulation was markedly suppressed (Bo *et al.*, 1991). Thus, we suggest that when progesterone or progestagens are used in conjunction with superovulation treatments, PGF_{2α} rather than oestradiol should be the luteolytic agent of choice to avoid effects on dominant follicle turnover.

The critical parameter when comparing differences in superovulation protocol is the number of freezable and transferable embryos produced. The significantly lower number of grade 1 and 2 embryos is a major limitation to the widespread use of a PRID as a method of synchronization in the absence of knowledge of the stage of the oestrous cycle at PRID insertion. The results of this experiment suggest a shift from grade 1 and 2 to grade 3 embryos when the PRID rather than PGF_{2α} was used. This finding agrees with the finding that norgestomet can affect oocyte quality (Maurer *et al.*, 1987); however, in another study (Hill *et al.*, 1986), stage of the cycle did not affect embryo quality and norgestomet appears to offer an alternative that results in successful superovulation in low fertility donors (Ellington *et al.*, 1987). Thus, the stage of the cycle, the presence of a dominant follicle, the dynamics of recruitment and selection of antral follicles and the health of the oocyte in the preovulatory follicle can all affect embryo production rates.

In superovulated cows, the fertilization rate is often as low as 65% and about 15% of superovulated cows have no fertilized ova (Hasler *et al.*, 1983). It is generally recommended that superovulated cows are inseminated twice with one straw at each insemination (West *et al.*, 1984). Some studies (Donaldson and Ward, 1985; Schiewe *et al.*, 1987) suggest that repeated inseminations are necessary to obtain optimal fertilization rates for the production of transferable embryos. In the study reported here, there was no difference in the number, or quality, of embryos recovered following the use of two or three straws at insemination, suggesting that the third straw of semen did not improve embryo quality. This is in agreement with data of Critser *et al.* (1980) who examined timing and number of inseminations used. The timing of ovulation in superovulated cows is variable (Yadav *et al.*, 1986) and repeated inseminations at intervals of 12 h are used in embryo transfer programmes. A high proportion of semen is lost from the reproductive tract within hours of insemination and this suggests that the site of semen deposition influences the distribution of spermatozoa and in turn influences the fertilization rate. In this study, there was a significant effect of site of semen deposition on the number of transferable embryos; uterine insemination yielded a higher number than when combined with cervical deposition. This finding does not support the hypothesis that the depletion of oviductal sperm reservoirs due to the protracted time of ovulation could be overcome by combining the routine intra-uterine insemination with simultaneous semen deposition in the cervix (Hunter, 1988). In the present study, thawed, frozen semen combined with PRID synchronization yielded the lowest number of freezable embryos and the highest number of degenerate embryos. This suggests an interaction with frozen semen and PRID synchronization that is not present with fresh semen.

In conclusion our data suggest that a PRID can be used successfully in a superovulatory regimen in cattle with due regard to the stage of the oestrous cycle at insertion. It is suggested that the dominant follicle has a major role in determining the superovulatory response. In addition, high quality thawed, frozen, or fresh semen must be used to ensure a high yield of good quality embryos suitable for freezing or fresh transfer. Two straws of semen are sufficient for insemination of superovulated heifers and there is no advantage in depositing an additional straw in the cervix.

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