In vivo oocyte recovery and in vitro embryo production from bovine oocyte donors treated with progestagen, oestradiol and FSH

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Abstract

The effect of treatment of donor cattle with progestagen and oestradiol or FSH on in vivo oocyte recovery and in vitro embryo production was studied. Forty-eight beef × Friesian cows formed eight replicates of six treatments in a 2 (no steroid versus steroid) × 3 (none, single or multiple dose(s) of FSH) factorial design in which follicles were aspirated once weekly for 3 weeks. Oocytes were graded, washed, matured for 20–24 h and then inseminated with frozen/thawed semen from a single sire followed by coculture on granulosa cell monolayers.

Treatment with steroid had no significant effect on any follicular, oocyte or embryo production variate other than to reduce the number (P < 0.05) and the diameter of large follicles > 10 mm (P < 0.01) present at aspiration. FSH increased numbers of medium (6–10 mm) and large follicles (P < 0.01) and there was a corresponding decrease in the number of small follicles (2–5 mm; P < 0.01). The total number of follicles at aspiration increased from 17.7 ± 1.09 for animals not treated with FSH to 23.6 ± 1.97 following multiple dose treatment with FSH (P < 0.05). Significantly, more follicles were aspirated following FSH treatment (no FSH 9.7 ± 1.09, single dose FSH 13.6 ± 1.30, multiple dose FSH 17.3 ± 1.52; P < 0.05) and numbers of oocytes recovered per cow per week increased (no FSH 4.1 ± 0.76, single dose FSH 5.3 ± 0.87, multiple dose FSH 5.9 ± 0.94) but the differences were not significant. Significantly, more good oocytes (Category 1) were recovered from animals treated with FSH (P < 0.05). There was no overall significant effect of FSH on embryo production rate or the total number of transferable embryos produced but the number of transferable embryos was highest following administration of multiple doses of FSH.

In conclusion, progestagen plus oestradiol 17β treatment did not affect follicle, oocyte and embryo production of oocyte donors aspirated once per week. FSH treatment, however, significantly...

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increased the number of follicles aspirated and Category 1 oocytes recovered. Multiple dose administration of FSH resulted in the production of the highest number of transferable embryos but this effect was not significant. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Cattle-reproductive technology; Oocyte; Embryo; Steroid; Gonadotrophin

1. Introduction

In vivo oocyte recovery using transvaginal ultrasound-guided follicular aspiration and in vitro embryo culture are established techniques for producing bovine embryos (Kruip et al., 1994; Looney et al., 1994; Hasler et al., 1995). These methods allow production of a greater number of viable embryos in a given period of time than conventional superovulation and in vivo embryo recovery (Looney, 1986; Tregaskes et al., 1996; Goodhand et al., 1997). They may be of particular benefit to beef MOET (multiple ovulation embryo transfer) genetic improvement schemes where only a short period of time is available for creation of embryos from selected donors if the theoretical contribution of a reduced generation interval to the improvement in the rate of genetic gain is to be achieved (Land and Hill, 1975; Smith, 1988; Broadbent, 1990).

Both an abundant supply of developmentally competent oocytes and an efficient method of in vitro embryo production are required in order to achieve the potential offered by these techniques. A number of different protocols for pretreatment of oocyte donors have been tried in an effort to improve oocyte yield and developmental competence following in vivo recovery. These include treatment with GnRH (Bordignon et al., 1996), gonadotrophin (Walton et al., 1993), gonadotrophin plus BST (Hwang et al., 1997) and anti-inhibin immunisation (Konishi et al., 1996). Treatment with FSH using different dosage regimes before ultrasound-guided follicular aspiration improves numbers of oocytes collected and embryos produced compared to unstimulated controls (Walton et al., 1993; Gibbons et al., 1994; Looney et al., 1994; Stubbings and Walton, 1995; Guyader et al., 1997; Goodhand et al., 1999). Single, rather than multiple, dose administration of gonadotrophin may have management advantages, for example, where animal restraint may cause stress (Bo et al., 1991; Tribulo et al., 1993). Consequently, one objective of the current experiment was to compare single versus multiple doses of FSH in terms of the number of follicles available for aspiration, oocytes recovered and transferable grade embryos produced.

Progestosterone and oestradiol have been administered at various stages of the bovine oestrous cycle in an attempt to induce regression of the dominant follicle and synchronised emergence of a new follicular wave (Bo et al., 1993; 1994a,b). The second objective of the current experiment was to determine whether administration of oestradiol benzoate with exogenous progesterone would improve the number of follicles available for aspiration in vivo, and the yield and developmental competence of the oocytes recovered.

2. Material and methods

2.1. Animals

Forty-eight cyclic beef × Friesian cows (mainly Hereford or Simmental crosses) were used as oocyte donors. Mean live weights and condition scores during the experimental
period were 588.2 ± 5.80 kg and 3.00 ± 0.08 units (scale of 1–5, where 1: very thin and 5: very fat; Lowman et al., 1976). The experiment was carried out during two periods (Periods 1 and 2) with a 4-week interval between periods. The animals were housed in straw-bedded pens. A diet of 3 kg concentrates (11.8 MJ ME/kg DM; 180 g CP/kg DM) plus 110 g mineral/vitamin supplement (Beef Cow; Norvite, UK) was fed daily and barley straw offered ad libitum in Period 1; and grass silage alone was offered ad libitum plus the same mineral/vitamin supplement in Period 2.

2.2. Treatments

Prior to treatment, oocyte donors had their oestrous cycles synchronised by an intravaginal device incorporating a 10 mg oestradiol benzoate capsule (PRID; 1.55 mg progesterone; Sanofi Animal Health, Watford, UK). This was followed by administration of prostaglandin (30 mg luprostiol; Prosolvin; Intervet (UK) Ltd, Cambridge, UK) 6 days later. The PRID was removed after a further 5 days to induce oestrus about 48 h later.

The experiment involved steroid (progestagen plus oestradiol 17β) and/or gonadotrophin (FSH) treatment in a 2 (no steroid versus steroid) × 3 (none, single or multiple dose(s) of FSH) factorial design in which each of the six treatments was replicated eight times. Replicates 1–4 and 5–8 were performed in Periods 1 and 2, respectively. Cows were ranked by condition score within parity and assigned at random to the six treatments with the randomisation restricted so that treatment groups were of similar condition score and parity at the start of the experiment. Each animal was used for oocyte recovery once weekly for 3 weeks. Treatment was administered on a weekly basis starting 2 days after the synchronised oestrus (Day 0). Half the animals remained untreated with steroids. On the steroid treatment, a 3 mg norgestomet ear implant (Crestar; Intervet (UK) Ltd, Cambridge, UK) was administered on Day 1 of each week. After aspiration on Day 4 of each week (Days 5, 12 and 19 after oestrus) a further 3 mg norgestomet (Intervet International, Boxmeer, Netherlands) in 2 ml sesame oil and 5 mg oestradiol 17β in 2 ml sesame oil (Sigma Chemical Company Limited, Little Chalfont, UK) were administered i.m. Within each of these steroid treatments there were three gonadotrophin treatments: no FSH, single dose FSH and multiple dose FSH. The single dose of FSH was given s.c. behind the shoulder as 9.0 mg NIADDK-oFSH-17β equivalent (Ovagen; ICP, Auckland, New Zealand) on Day 1 of each week (Days 2, 9 and 16 after oestrus). The multiple doses of FSH were administered i.m. twice daily on Days 1, 2 and 3 of each week (Days 2–4, 9–11, and 16–18 after oestrus) in a declining pattern of 2 (2.02 + 1.75 + 1.00) mg = 9.0 mg in total.

2.3. Follicular aspiration and oocyte recovery

Follicular aspiration and oocyte recovery were on Day 4 of the weekly programme and all first aspirations were on Day 5 after oestrus. Not all cows were aspirated on the same day of the week but all treatments were represented on each day of aspiration in Period 1 and steroid and no steroid treatments were represented on consecutive days in Period 2.

Prior to oocyte collection, all cows received an epidural anaesthetic of 5–10 ml lignocaine hydrochloride (Locovetic; SmithKline Beecham, Tadworth, UK); and intravenous administrations of 5 mg acepromazine (ACP; C-Vet, Bury St. Edmunds, UK) and 5 mg detomidine
hydrochloride (Domosedan; SmithKline Beecham, Tadworth, UK). The ovaries were visualised using a 5 MHz transvaginal curvilinear ultrasound transducer (Aloka SSD-500 V; BCF, Livingston, UK) and all visible follicles were categorised by size as 2–5 mm, 6–10 mm or >10 mm in diameter. All accessible follicles ≥2 mm diameter were aspirated using a 55 cm, 20 g single lumen needle. A vacuum of 70–80 mmHg was applied by means of a pump (Craft Duo-vac; Rocket, Watford, UK) during aspiration. The needle was withdrawn at intervals and rinsed with a solution of Dulbecco’s PBS supplemented with 0.3% (v/v) heparin, 1% (v/v) fetal calf serum (FCS) and antibiotics (50 IU/ml penicillin, 50 μg/ml streptomycin and 100 μg/ml neomycin). Following each oocyte recovery, donors received 15 ml penicillin i.m. (150 mg/ml procaine penicillin, 112.5 mg/ml benzathine penicillin; Duphapen LA; Solvay-Duphar Veterinary, Southampton, UK). The aspiration rate was calculated as the number of follicles aspirated expressed as a percentage of the total number of follicles counted for each cow.

Aspirants were collected in 50 ml centrifuge tubes kept at a temperature of 37°C in a heated block. The contents of the tube were searched for oocytes within 30 min of collection. The oocyte recovery rate was calculated as the number of oocytes recovered expressed as a percentage of the number of follicles aspirated for each cow. Oocytes were classified into four categories according to the nature of the cumulus and cytoplasm: Category 1: four layers of compact, light or transparent cumulus with clear, even cytoplasm; Category 2: compact, <4 layers cumulus and/or cytoplasm generally homogenous but with a coarser appearance; Category 3: <4 layers of cumulus and/or cytoplasm of irregular appearance with dark areas, cumulus and oocyte generally darker than first two categories or cumulus may be expanded; Category 4: no cumulus.

2.4. In vitro embryo production

Oocytes in Categories 1–3 were matured in groups of 1–20 in 2 ml culture medium (TCM 199 plus 10% (v/v) steer serum, 50 IU/ml penicillin, 50 μg/ml streptomycin) supplemented with 2–6 × 106 granulosa cells at a temperature of 39°C in an atmosphere of 5% CO2 plus air and 100% humidity for 20–24 h. Oocytes in Category 4 were not used. Following maturation, oocytes were washed and expanded cumulus cells were reduced to 4 or 5 layers by careful pipetting. The oocytes were then rinsed again and placed in groups of 1–20 in 46 μl drops of fertilisation medium (TALP supplemented with 0.6% (v/v) BSA, 50 IU/ml penicillin, 50 μg/ml streptomycin).

Frozen/thawed semen from a single Simmental sire with good performance characteristics and which gave good results in the in vitro embryo production system used here was used for insemination. Motile sperm, obtained by swim-up separation of frozen/thawed semen, were capacitated using heparin (30 μg/ml) and motility was stimulated by addition of 40 μl/ml PHE (penicillamine 20 μM, hypotaurine 10 μM and epinephrine 1 μM). Approximately, 50,000 live sperm in 4 μl fertilisation medium were added to each fertilisation drop and the oocytes were incubated at 39°C, in 5% CO2 in air and 100% humidity for 20–24 h. Fertilised ova were rinsed in culture media and pipetted in groups of 1–20 on to granulosa cell monolayers in 50 μl drops of culture medium under sterile mineral oil. Cumulus cells were stripped off early embryos 48 h after insemination and the number of cleaved oocytes counted. The cleavage rate was calculated as the number of cleaved oocytes expressed as
a percentage of the number of oocytes placed in maturation medium. On the fourth day of culture, half of the medium in the drop was removed and replaced with fresh culture medium. Embryos were counted and evaluated on Day 7 after insemination and graded as follows: 1: excellent, 2: good, 3: fair, 4: poor (Lindner and Wright, 1983). The embryo production rate was calculated as the number of transferable embryos (Grades 1 and 2) produced expressed as a percentage of the number of oocytes used.

In Period 1 (replicates 1–4), the oocytes from two or more cows were combined for in vitro embryo production irrespective of treatment to avoid the outcome being affected by culturing small numbers of oocytes in one culture drop. In Period 2 (replicates 5–8), oocytes from pairs of cows on the same treatment were cultured together. Consequently, only embryos produced in replicates 5–8 were included in the results. Chi-square analysis of the embryo production data showed that there were no significant differences between the overall results for Periods 1 and 2.

2.5. Statistical analyses

Follicle numbers, oocyte recovery and embryo evaluation data were analysed using Generalised Linear Models (McCullagh and Nelder, 1989) which allow the effect of several factors to be studied simultaneously. Cow, treatment, week and period of aspiration were included as factors in the model. A heterogeneity factor was estimated and used to adjust the S.E.M. Data were expressed as means per cow per week ± S.E.M. The significance of differences between means was evaluated by t-test.

3. Results

There were no statistically significant interactions between the main effects (steroid and gonadotrophin pretreatment) and no significant effects of week of aspiration or week of in vitro culture (weeks 1–3 within animal) for any of the variates studied. Consequently, only the mean data for main effects expressed on a per cow per week basis are presented.

Treatment with steroid had no significant effect on numbers of follicles observed or aspirated, oocytes recovered or cleaved, oocyte quality, number of transferable embryos; or on aspiration, recovery or embryo production rates (Tables 1–4). Cows which received steroid treatment had fewer large follicles (0.9±0.13 versus 1.6±0.17; P < 0.05; Table 1), and the mean diameter and range in sizes of the largest follicle (11.0 ± 0.25 mm versus 12.7 ± 0.40 mm, P < 0.01; and 7–19 mm versus 8–33 mm, respectively) were smaller than for cows not treated with steroids.

Treatment with FSH significantly increased numbers of medium (6–10 mm) and large (>10 mm) follicles and there was a corresponding decrease in the number of small (2–5 mm) follicles (P < 0.01; Table 1). Multiple doses of FSH produced more ovarian follicles greater than 10 mm diameter than single dose FSH (2.0 ± 0.23 versus 1.1 ± 0.18; P < 0.01). The total number of follicles increased from 17.7 ± 1.60 with no FSH to 20.3 ± 1.73 with single dose FSH and 23.6 ± 1.97 following multiple dose FSH. The difference between multiple dose and no FSH treatment was significant (P < 0.05; Table 1).
Table 1
Number of follicles available for aspiration (mean per cow per week ± S.E.M.; eight replicates per treatment)

<table>
<thead>
<tr>
<th>Follicle diameter</th>
<th>Pharmacological treatment</th>
<th>Level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Steroid</td>
<td>Gonadotrophin (FSH)</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>Progestagen + oestradiol</td>
</tr>
<tr>
<td>2 to 5 mm</td>
<td>9.4 ± 0.86</td>
<td>11.4 ± 0.95</td>
</tr>
<tr>
<td>6 to 10 mm</td>
<td>9.2 ± 0.89</td>
<td>7.9 ± 0.89</td>
</tr>
<tr>
<td>&gt;10</td>
<td>1.6 ± 0.17</td>
<td>0.9 ± 0.13</td>
</tr>
<tr>
<td>Total</td>
<td>20.4 ± 1.43</td>
<td>20.6 ± 1.49</td>
</tr>
</tbody>
</table>
Table 2
Effect of treatment on numbers of follicles aspirated and oocytes recovered (mean per cow per week ± S.E.M.; eight replicates per treatment)

<table>
<thead>
<tr>
<th>Pharmacological treatment</th>
<th>Level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroid</td>
<td>Gonadotrophin (FSH)</td>
</tr>
<tr>
<td>None</td>
<td>Progestagen + oestradiol</td>
</tr>
<tr>
<td>Follicles aspirated</td>
<td>13.3 ± 1.06</td>
</tr>
<tr>
<td>Aspiration rate</td>
<td>0.65 ± 0.03</td>
</tr>
<tr>
<td>Total oocytes recovered</td>
<td>4.9 ± 0.68</td>
</tr>
<tr>
<td>Oocyte recovery rate</td>
<td>0.37 ± 0.03</td>
</tr>
<tr>
<td>Oocyte category</td>
<td>Pharmacological treatment</td>
</tr>
<tr>
<td>-----------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td></td>
<td>Steroid</td>
</tr>
<tr>
<td></td>
<td>None</td>
</tr>
<tr>
<td>1</td>
<td>2.3 ± 0.36</td>
</tr>
<tr>
<td>2</td>
<td>1.2 ± 0.23</td>
</tr>
<tr>
<td>3</td>
<td>1.2 ± 0.22</td>
</tr>
<tr>
<td>4</td>
<td>0.2 ± 0.06</td>
</tr>
</tbody>
</table>
### Table 4

Effect of treatment on in vitro embryo production by Day 7 after insemination (mean per cow per week ± S.E.M.; four replicates per treatment)

<table>
<thead>
<tr>
<th>Pharmacological treatment</th>
<th>Gonadotrophin (FSH)</th>
<th></th>
<th>Level of significance</th>
<th>Steroid vs. no steroid</th>
<th>FSH vs. no FSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroid</td>
<td>None</td>
<td>Progestagen + oestradiol</td>
<td>None</td>
<td>Single dose</td>
<td>Multiple dose</td>
</tr>
<tr>
<td>Oocytes used</td>
<td>5.0 ± 0.46</td>
<td>6.1 ± 0.51</td>
<td>4.7 ± 0.55</td>
<td>5.2 ± 0.58</td>
<td>6.8 ± 0.66</td>
</tr>
<tr>
<td>Oocytes cleaved</td>
<td>3.7 ± 0.52</td>
<td>4.5 ± 0.58</td>
<td>3.5 ± 0.63</td>
<td>3.7 ± 0.64</td>
<td>5.1 ± 0.75</td>
</tr>
<tr>
<td>Cleavage rate</td>
<td>0.72 ± 0.05</td>
<td>0.74 ± 0.05</td>
<td>0.74 ± 0.07</td>
<td>0.69 ± 0.07</td>
<td>0.76 ± 0.05</td>
</tr>
<tr>
<td>Transferable embryos</td>
<td>1.8 ± 0.25</td>
<td>2.4 ± 0.35</td>
<td>2.0 ± 0.38</td>
<td>1.9 ± 0.35</td>
<td>2.5 ± 0.40</td>
</tr>
<tr>
<td>Embryo production rate</td>
<td>0.37 ± 0.05</td>
<td>0.38 ± 0.04</td>
<td>0.43 ± 0.06</td>
<td>0.33 ± 0.05</td>
<td>0.37 ± 0.05</td>
</tr>
</tbody>
</table>
Table 2 shows that treatment with FSH significantly increased the number of follicles aspirated \( (P < 0.05) \) and the aspiration rate \( (P < 0.01) \) compared to no FSH. These effects were greater for multiple compared to single dose FSH treatment but the difference between them was significant only for aspiration rate \( (P < 0.05) \). The total number of oocytes recovered increased from no FSH to single to multiple treatment and, conversely, recovery rate tended to decrease but these effects were not significant \( (P > 0.05; \text{Table 2}) \). Treatment with FSH, however, did improve the quality of oocytes recovered and significantly more oocytes from this treatment were graded Category 1 \( (P < 0.05; \text{Table 3}) \). There was no significant effect of FSH treatment on the numbers of oocytes in Categories 2, 3 or 4.

The number of oocytes which cleaved by 48 h after insemination and the cleavage rate were not significantly affected by FSH treatment, and there was no significant effect of FSH treatment on embryo production rate or the total numbers of transferable embryos produced \( (\text{Table 4}) \). The highest numbers of transferable embryos were produced following multiple dose FSH but the difference was not significant.

4. Discussion

Exogenous progesterone suppresses the dominant follicle when administered during its growing phase and leads to early emergence of the next follicular wave \( (\text{Adams et al., 1992}) \). Oestradiol also induces atresia and oestradiol valerate given in the early growing phase of the dominant follicle terminates its growth and functional dominance \( (\text{Bo et al., 1992, 1993}) \). Oestradiol 17\( \beta \) was used in this experiment and combined with progesterone because this treatment was reported to give the most consistent suppression of the dominant follicle and synchronised emergence of a new follicular wave \( (\text{Bo et al., 1993; 1994a,b}) \).

The pattern of follicular wave development prior to treatment was not established for the animals used in this experiment. It has been reported, however, that most animals exhibit either two or three waves with wave emergence occurring on Day 0 and 10 for two wave cycles, and on Days 0, 9 and 16 for three wave cycles \( (\text{Ginther et al., 1989a,b}) \). Aspiration of all follicles \( \geq 2 \text{ mm diameter} \) would be expected to over ride any intrinsic follicular wave pattern \( (\text{Bergfelt et al., 1994}) \) but the experiment did not allow a follicular wave pattern to be established and detected by ultrasound. If, however, aspiration had not interfered with the normal wave pattern then administration of the norgestomet implants would have coincided, in most cows, with the early growth phase of a new wave \( (\text{Days 2, 9 and 16 in three wave cycles; Days 2 and 10 in two wave cycles}) \). The subsequent combined steroid treatment would have been expected to coincide with the early to late growing phase of the follicular wave and to induce synchronous wave emergence in these animals \( (\text{Bo et al., 1994a}) \). Steroid treatment had the anticipated effect of causing significant reductions in the number and diameter of large follicles \( (\text{Adams et al., 1992}) \) but there was no significant effect on any other characteristic of the follicles. This implies that aspiration controlled the course of folliculogenesis in these animals and the steroid treatment did not affect the development of new follicular waves.

Gonadotrophin treatment initiated on the day of, or the day before, wave emergence has been shown to produce a high superovulatory response \( (\text{Adams et al., 1993; Nasser et al., 1993}) \). Consequently, FSH treatment was timed to coincide with expected wave
emergence 4 days after progestagen plus oestradiol 17β administration. FSH treatment increased numbers of medium sized follicles seven-fold and large sized follicles three-fold with a corresponding decrease in numbers of small follicles. Similar results have been reported previously (Lonergan et al., 1993; Goodhand et al., 1999). Gonadotrophin treatment increased the number of follicles available for aspiration, the aspiration rate and the number of oocytes recovered in agreement with other reports (Pieterse et al., 1988, 1992; Looney et al., 1994; Stubbings and Walton, 1995; Goodhand et al., 1999).

Multiple dose FSH stimulated ovarian activity more effectively, without compromising oocyte quality, than a single dose (Stubbings et al., 1993; Walsh et al., 1993) unless the single dose FSH was accompanied by a low dose of PMSG (Irvine et al., 1993; Armstrong et al., 1994). In the present experiment, multiple dose FSH in the absence of exogenous steroids had the greatest effect on follicle size distribution. There was no difference between single and multiple dose FSH in the presence of exogenous steroids, probably because the steroids caused the early demise of growing-phase dominant follicles and an early surge in endogenous FSH (Adams et al., 1992; Bo et al., 1993).

The route of administration, and frequency, may affect the relative effect of single or multiple dose FSH. Twice daily i.m. administration of FSH gave higher superovulatory responses than the same total given s.c. as one (Walsh et al., 1993) or two (Bo et al., 1991) injections per day. However, a single s.c. administration of FSH resulted in a superovulatory response similar to a twice daily i.m. regimen for 4 days (Bo et al., 1991; Staigmiller et al., 1992). The response was more consistent when the single s.c. injection was behind the shoulder, as in the current experiment, rather than the neck (Bo et al., 1991; Hockley et al., 1992).

Increased oocyte quality has been associated with increased follicle size and oocyte developmental potential following FSH treatment (Lonergan et al., 1992; 1993; Arlotto et al., 1996; Goodhand et al., 1999). Not all studies agree that these are the consequences of FSH stimulation (Blondin and Sirard, 1994; Blondin et al., 1995) since oocyte and follicle maturation may be uncoupled (de Loos et al., 1991; Blondin et al., 1996). This causes asynchrony between the nuclear and cytoplasmic components of oocyte maturation (Bousquet et al., 1995) and disturbed oocyte development. However, embryo production rate in the present study was not affected, in agreement with the report by Gibbons et al. (1994), and number of embryos produced tended to be higher following FSH treatment as we observed previously (Goodhand et al., 1999).

5. Conclusions

Treatment of oocyte donors with steroids (progestagen plus oestradiol 17β) did not affect follicle numbers, oocyte recovery or embryo production. The effect of administering FSH was to increase the number of follicles aspirated and Category 1 oocytes recovered; and the highest numbers of transferable embryos were produced when multiple doses of FSH were administered. Considered in conjunction with information published in the literature, it is concluded that gonadotrophin (FSH) treatment administered in multiple doses prior to aspiration is likely to improve the numbers of follicles available for aspiration, follicles aspirated, and both the numbers and quality of oocytes and embryos recovered or produced.
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