Use of bovine oocytes for the evaluation of ram semen

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ABSTRACT

In vitro fertilisation (IVF) is one of a range of procedures used to evaluate the fertility of ram semen. This paper reports on the development of a heterologous IVF test, using bovine oocytes for the evaluation of frozen ram semen. An initial comparison of frozen bull (B) and ram (R) sperm with bovine oocytes using either TALP or SOF media showed a significant ($P<0.001$) species by media interaction on the percentage of fertilised oocytes (B-TALP 88.2±1.6; B-SOF 9.8±4.2; R-TALP 21.3±7.0; R-SOF 52.0±12.2). Subsequent evaluations of B-TALP and R-SOF in 9 trials with bovine oocytes gave values of 84.4±2.3 and 67.9±4.8 respectively.

Comparisons of ewe and cow oocytes with both B-TALP and R-SOF have provided very similar results, thus allowing the interchange of oocytes for testing sperm of both species.

The proportion of bovine oocytes fertilised showed a curvilinear dose response to the concentration of ram sperm in the IVF media.

Frozen semen from 19 individual rams has been evaluated using this system and there was a significant ($P<0.01$) between ram effect. The percentage of fertilised oocytes has ranged from 8.0±2.0 to 89.9±0.4 for individual rams, while those for six different ejaculates from the same ram ranged from 61.5±13.0 to 87.5±3.2, with a significant between ejaculate effect ($P<0.05$).

Keywords: ram semen; IVF; bovine oocytes; heterologous IVF.

INTRODUCTION

The use of *in vitro* fertilisation (IVF) techniques to determine the fertilising ability of sperm is one of the parameters for the laboratory evaluation of ram sperm viability (fertility). Normally the IVF procedures use oocytes and sperm from the same species. Obtaining a continual supply of ewe oocytes for IVF, from ovaries collected at abattoirs, on a year round basis is a major problem at Ruakura. However, such a regular supply of cow oocytes is available and used in our embryology laboratory for IVF of bull semen.

Heterologous IVF between bovine and ovine gametes has been previously reported (Slavik *et al.*, 1990 and Cox *et al.* 1993). This paper describes our attempts to develop a routine IVF test for frozen ram semen based on the use of cow oocytes.

METHODS

Media:

The following media preparations were used and the concentrations expressed are final values for that media.

IVM = tissue medium (TCM 199) + 25 mM $NaHC0_3$ + 1 mM glutamine + 0.2 mM pyruvate + 0.01 unit/ml Ovagen (FSH) + 10% foetal calf serum + 10 µg/ml oLH + 1 µg/ml E2-17β.

HSOF = synthetic oviduct fluid (SOF - Tervit et al. 1972) + 20.0 mM HEPES.

SOF-IVF = SOF + amino acids + 2% OES (oestrus ewe serum) + 0.02 mM penicillimide + 0.01 mM hypotaurine.

HTALP = TALP (Parrish *et al.* 1988) + 20 mM HEPES.

TALP-IVF = TALP + 0.01 mg/ml heparin + 0.02 mM penicillimide + 0.01 mM hypotaurine.

IVC = SOF + amino acids + 0.8% BSA.

Oocyte collection and *in vitro* maturation (IVM):

Cow or ewe ovaries were collected at the abattoir, placed in a warm saline solution and transferred to the laboratory. Follicles were aspirated and oocytes placed in IVM media (10 oocytes/50 µl drop under oil for 24 h at 39°C in 5% CO_2 in air).

In vitro fertilisation:

Matured oocytes were washed in either TALP-IVF for use with bull semen or SOF-IVF for ram semen and placed into pre-equilibrated 30 µl drops (5 oocytes in 10 µl/drop) at 39°C in 5% CO_2 in air for 24 h (30-40 oocytes were allocated to each treatment group ie. 6 to 8 IVF drops per treatment).

Semen preparation:

Frozen bull or ram semen was thawed at 30°C, layered onto percoll, centrifuged, washed once with either 50 µl HTALP (bull) or HSOF (ram), resuspended and adjusted to final concentration of 5 or 10 x 10^6 sperm/ml. Sperm suspension (10 µl) was added to the oocytes (40 µl drops) to give final concentration of 1 or 2 x 10^6/ml.

In vitro culture:

After the 24 h IVF procedure the oocytes were placed in IVC under the same conditions for a further 24-48 h.

Oocyte staining: Initially, at completion of IVF oocytes were fixed in a 1:3 v/v acetone-ethanol mixture for 48 h, stained with acetolacmoid and were considered fertilised

when pro-nuclear development and a sperm tail could be observed. Because of time constraints this was changed and subsequently, after IVC all eggs were stained with H33342, examined under UV fluorescence microscopy and the presence of 2 or more nuclei was taken as indication of fertilisation.

Experimental protocol:

A series of 5 experiments were conducted. Details of the protocols are given in the results section.

Statistical analysis:

The proportion of oocytes classed as fertilised to those inseminated (fertilisation rate) was analysed using Chi square tests. In Experiment 4 the linear and quadratic polynomial effects of semen concentration were determined.

RESULTS (AND EXPERIMENTAL PROTOCOL)

Experiment 1:

An initial comparison of frozen bull and ram sperm was made using both sources of sperm with cow oocytes in each of two media (TALP and SOF +2% OES). The experiment was repeated 4 times and used a total of 430 oocytes.

There was a significant ($P<0.001$) interaction between semen species and media (Table 1).

TABLE 1: Effect of IVF media on the fertilisation rate of cow oocytes with bull or ram semen. (Values are means ± sem from 4 trials).

Media	Bull Semen	Ram Semen
TALP	88.2 ± 1.6	21.3 ± 7.0
SOF + 2% OES	9.8 ± 4.2	52 ± 12.2

Experiment 2:

Comparisons of the repeatability of fertilisation of cow oocytes with either bull sperm in TALP-IVF or ram sperm in SOF + 2% OES medium were made over 9 trials using semen from 1 bull and 1 ram and involving a total of 630 oocytes.

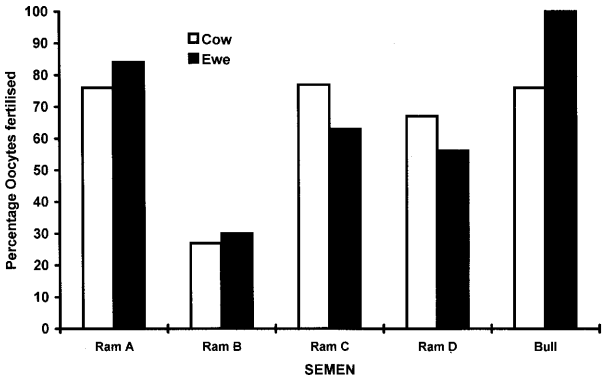
The fertilisation rate was higher ($P<0.05$) with bull semen (84.4 ± 2.3) than with ram semen (67.9 ± 4.8).

Experiment 3:

Comparisons of the fertilisation of either ewe or cow oocytes with either ram or bull semen were made. The bull semen was from the same batch as used in the previous experiments, while semen from 4 rams (3 new rams + the one used previously) was tested. Oocytes of either species were placed in TALP for insemination with bull semen and in SOF + 2% OES for insemination with ram sperm. The experiment was repeated in two trials and a total of 300 oocytes of each species was involved.

There was no significant difference between the species of oocytes in fertilisation rate either overall or within individual sources of semen. There was a significant ($P<0.01$) difference in fertilisation rate between rams but the overall ram versus bull comparison was not significantly different with cow oocytes but approached significance ($P<0.1$) with the ewe oocytes favouring the bull sperm (Figure 1).

FIGURE 1: Effect of source of oocytes (cow □ or ewe ■) on the fertilisation rate of semen from 4 rams and a bull. (Values are means from two tests).



Experiment 4:

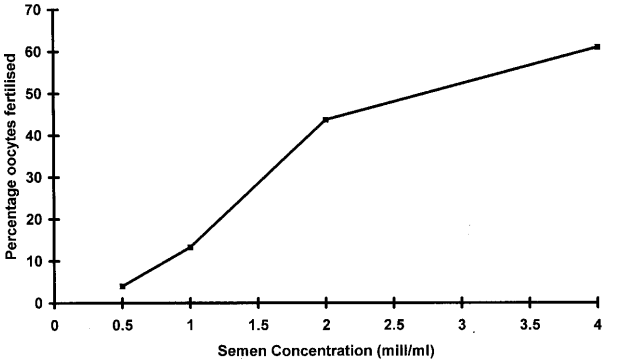
This experiment examined the effect of concentration of ram sperm in the IVF procedure on fertilisation of cow oocytes. Four concentrations of ram sperm 0.5, 1.0, 2.0 and 4.0 x 10^6/ml were used in each of two tests using 350 oocytes (a sample of bull semen at 1.0 x 10^6/ml was included as a control).

Fitting a log linear regression (logit) on the percentage of oocytes fertilised showed an ordered dose response effect of semen concentration with significant linear ($P<0.001$) and quadratic ($P<0.05$) components. The curvilinear nature of the effect is shown in Figure 2.

Experiment 5(a):

This experiment compared the fertilisation of cow oocytes with a single batch of semen from each of 19 individual rams: Each test involved the comparison of 4 unknown samples of semen together with two control samples (the standard bull semen used in the previous experiments and a ram semen standard prepared from

FIGURE 2: Effect of concentration of ram semen in the IVF medium on the fertilisation rate of cow oocytes.



freezing a large batch of semen pooled from 16 rams). Each comparison was repeated on at least two occasions. The experiment involved 10 separate trials and 1800 oocytes.

There was a significant ($P<0.001$) effect of individual rams whose fertilisation rates ranged from 8.0 ± 2.0 to 89.9 ± 0.4.

Experiment 5(b):

Semen from six different ejaculates collected over a period of 10 months from the same ram were evaluated using the same procedure as in 5(a) with two controls and 3 samples per test. Four tests were performed using a total of 600 oocytes.

There was a significant ($P<0.05$) difference in the fertilisation rates between ejaculates (batches) from the one ram which ranged from 61.5 ± 13.0 to 87.5 ± 3.2 ($P<0.05$).

DISCUSSIONS AND CONCLUSIONS

These data confirm the ability of bovine and ovine gametes to undergo heterologous fertilisation under in vitro conditions. The interaction between semen species and the IVF media seen in Experiment 1 indicates the different requirements for capacitation and maintenance of sperm viability in the two species, while the results of latter experiments indicate that the oocytes appeared to perform equally well in either IVF medium. This is further supported by the results of experiment 3 where both ewe and cow oocytes performed similarly in either IVF media.

The effect of ram sperm concentration in the IVF procedure with cow oocytes is similar to that seen with homologous IVF in either species. A standard dose of 2.0×10^6 sperm is now being used in our assays, although, where possible, two dose levels (1.0 and 2.0×10^6/ml) are used because more recent results (not presented) have indicated differences between rams in the shape of the dose response curve.

The similar performance of both ewe and cow oocytes when fertilised by either bull or ram semen indicates that the use of a heterologous IVF procedure to determine the fertilising ability of a semen sample is a valid and viable procedure. However, our results do differ from those reported by Slavik *et al.* (1990) who found significantly lower fertilisation rate in cow oocytes than in ewe oocytes when incubated with ram sperm in an IVF system.

The very large between ram and between ejaculate within ram results obtained with heterologous IVF in these trials has also been seen in conception rates to artificial insemination in other trials (Smith *et al.*, 1995a,b). Research is currently underway to correlate the IVF performance of semen batches with conception rates following the intrauterine insemination of the semen.

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