IN VITRO BOVINE EMBRYOS PRODUCTION: INFLUENCE OF SERUM AND HORMONAL SUPPLEMENTATION

PRODUCCIÓN IN VITRO DE EMBRIONES DE BOVINO: INFLUENCIA DE LA SUPLEMENTACIÓN SÉRICA Y HORMONAL

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SUMMARY

On study the effect of different serum and hormonal supplements, when added to the maturation medium, on the in vitro maturation and subsequent fertilization and cleavage process of bovine oocytes. The oocyte maturation, fertilization and cleavage rates in the presence of ECS, FCS and hormonal supplements were significantly higher (p<0.001) than that obtained in the control group. No significant differences for maturation, fertilization and development were observed between the different supplements. The addition of serum and hormonal supplements to the maturation medium improved the in vitro maturation rates and subsequent in vitro fertilization and cleavage rates of bovine oocytes.

RESUMEN

El objetivo del presente estudio fue investigar si diferentes suplementos séricos y hormonales, cuando se adicionan al medio de maduración, mantienen los procesos de maduración, fecundación y división de ovocitos de bovino in vitro. Los índices de maduración ovocitaria, fecundación y división, en presencia de los suplementos ECS, FCS y hormonas fueron superiores significativamente (p<0,001) que aquellos observados en el grupo control. No se apreciaron diferencias significativas para los estadíos de maduración, fecundación y división entre los diferentes suplementos estudiados. Concluimos que la adición de suplementos séricos y hormonales al medio de maduración mejora los índices de maduración ovocitaria y subsiguientes índices de fecundación y división in vitro de ovocitos de bovino.

INTRODUCTION

Techniques for producing pre-implantation embryos by in vitro

maturation/in vitro fertilization (IVM/IVF) are being used in many laboratories worldwide. Viable embryos can be produced from ovarian oocytes collected hours after death of animals at the slaughterhouse. It was reported that hormonal and/or follicular factors are needed to promote cytoplasmic maturation. Unfortunately, in vitro matured bovine oocytes have lower fertilizability and developmental capacity to the blastocyst stage than in vivo-matured oocytes, although several investigators have produced offspring from oocytes cultured in vitro (Fukuda et al., 1990). These results suggest that failure in previous attempts of fertilization and further development of oocytes matured in vitro were due to inadequate oocyte culture systems. Several attempts have been made to modify the culture conditions, including the addition of hormones, sera and granulosa cells to the maturation medium for in vitro oocyte maturation, but the percentage of oocytes reaching the blastocyst stage with in vitro culture systems is still low (Gliedt et al., 1996).

The objective of this study was to evaluate the effects of two different sera and two hormonal supplements on the in vitro maturation and subsequent fertilization and cleavage of bovine oocytes.

MATERIALS AND METHODS

The maturation, fertilization and culture procedures were made as described by Kim et al. (1990).

EXPERIMENTAL DESIGN

The oocytes were matured for 24 h according to the following scheme: (1) a group of oocytes was cultivated in basic maturation medium supplemented with 20 percent ECS; (2) other group of oocytes was matured in the presence of 20 percent FCS; (3) a group of oocytes was matured in TCM-199 medium supplemented with 4 IU/ml PMSG+2 IU/ml hCG (Laboratorios Ovejero, León, España); (4) the last group of oocytes was matured in the basic maturation medium with no supplements, serving as control group. After maturing period, some oocyte were cytogenetically evaluated by studying the meiotic maturation stage, the remaining oocytes were fertilized and subsequently cultured for developing.

After 72 h in culture, presumptive zygotes were cytogenetically evaluated. Data were statistically analysed by Chi-square test.

RESULTS AND DISCUSSION

As is shown in table I, when follicular oocytes were matured in TCM-199 medium supplemented with ECS, FCS or hormonal supplements the maturation rates were significantly higher (80.4 percent, 78.3 percent and 85.1 percent respectively, \( p<0.001 \)) than those obtained in control group (51.9 percent). There were no significant differences between the maturation percentages obtained in supplemented oocyte groups.

In addition, the percentages of oocytes matured in the presence of ECS, FCS or hormonal supplements which reached the two-pronuclei stage (fertilized oocytes) were significantly
Table I. The influence of different sera and hormonal supplements added to the maturation medium on in vitro maturation and subsequent fertilization and cleavage of bovine oocytes.

<table>
<thead>
<tr>
<th>Types of supplements</th>
<th>Number of oocytes cultured</th>
<th>Number of oocytes matured*</th>
<th>Number of oocytes inseminated</th>
<th>Number of ova fertilized*</th>
<th>Number of ova cleaved*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECS</td>
<td>250</td>
<td>80.4±4.7(^a)</td>
<td>250</td>
<td>53.7±4.4(^a)</td>
<td>57.7±3.7(^a)</td>
</tr>
<tr>
<td>FCS</td>
<td>250</td>
<td>78.3±4.5(^a)</td>
<td>250</td>
<td>50.4±3.1(^a)</td>
<td>55.4±3.7(^a)</td>
</tr>
<tr>
<td>PMSG+hCG</td>
<td>250</td>
<td>85.1±5.1(^a)</td>
<td>250</td>
<td>57.1±3.7(^a)</td>
<td>60.1±4.0(^a)</td>
</tr>
<tr>
<td>Control</td>
<td>250</td>
<td>51.9±3.2(^b)</td>
<td>250</td>
<td>32.7±2.9(^b)</td>
<td>20.4±2.0(^b)</td>
</tr>
</tbody>
</table>

\(^a,b\) Different superscripts in the same column denote significant differences when compared by Chi-square test (p<0.001). Data are showed as mean percentages±SEM from 5 replicates.

higher (53.7 percent, 50.4 percent, and 57.1 percent respectively; p<0.001) than those found in control group (32.7 percent). No significant differences between ECS, FCS and hormonal treatments were found.

Finally, the percentages of cleaved ova in the presence of ECS (57.7 percent), FCS (55.4 percent) or PMSG+hCG (60.1 percent) were significantly higher (p<0.001) than those observed in control group (20.4 percent). No significant differences were observed between the supplemented oocytes groups.

The results obtained in our study demonstrated the important role that serum and hormonal supplementation plays in the maturation and subsequent fertilization and cleavage process supporting our studies (Gliedt et al., 1996). It is suggested that serum and hormonal supplements contain factors which stimulate in vitro maturation, fertilization and subsequent embryonic development (Mattioli et al., 1991; Schroeder et al., 1991).

The ECS supplement is widely used in the maturation medium of preovulatory bovine oocytes (Sanbuissho and Threlfall, 1990), in vitro fertilization (Kim et al., 1990) and subsequent embryonic development (Lambert et al., 1986). Our results are similar to those reported by Schellander et al. (1990), who postulated that the beneficial effects of ECS supplement might be due to the relatively high LH content. This hormone is known to be responsible for breaking the COC-oocyte interaction and activating maturation process (Saeki et al., 1990).

FCS is an important serum supplement widely used in the oocyte maturation and fertilization in vitro. It was postulated that the beneficial effect of FCS might be due to maturation-stimulating components such as hormones, proteins and growth factors, similar to those found in ECS supplement. These stimulating factors are active on the germinal vesicle breakdown inducing the meiotic...
resumption of the immature oocytes (Mattioli et al., 1991).

It has been reported that FCS contains some undefined growth-promoting components that are absent in the serum of adult animals (Mochizuki et al., 1991).

Finally, it has been reported that hormonal supplementation is necessary for activating both cytoplasmic and nuclear oocyte maturation mechanism, as well as in vitro fertilization and embryonic development (Sanbuisho and Threlfall, 1990; Brackett and Zuelke, 1993). These hormones are known to contain high LH hormone levels.

In conclusion, the addition of serum or gonadotropin hormones to the maturation medium improved the meiotic maturation and subsequent fertilization and cleavage rates of bovine oocytes matured and fertilized in vitro.

REFERENCES


Archivos de zootecnia vol. 48, núm. 181, p. 74.