

NOTA BREVE

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**

Ocaña Quero, J.M.^{1*}, M. Moreno Millán¹, M. Pinedo Merlin², M.A. Ortega Mariscal³ y A. Rodero Franganillo¹

¹Departamento de Genética. Facultad de Veterinaria. 14005-Córdoba.España.

²Departamento de Patología Animal. Facultad de Veterinaria. 14005-Córdoba. España.

³Departamento de Bromatología y Tecnología de los Alimentos. Campus de Rabanales. Córdoba. España.

ADDITIONAL KEYWORDS

Bovine oocyte. *In vitro* maturation. *In vitro* fertilization. Serum. Gonadotropin hormones.

PALABRAS CLAVE ADICIONALES

Ovocito bovino. Maduración *in vitro*. Fecundación *in vitro*. Suero. Hormonas gonadotrópicas.

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.

monales, 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 estadios 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.

RESUMEN

El objetivo del presente estudio fue investigar si diferentes suplementos séricos y hor-

*Correspondencia

INTRODUCTION

Techniques for producing pre-implantation embryos by *in vitro*

Arch. Zootec. 48: 71-74. 1999.

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

IN VITRO BOVINE EMBRYOS PRODUCTION

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. (Influencia de diferentes suplementos séricos y hormonales añadidos al medio de maduración sobre la maduración *in vitro* y posterior fecundación y división de ovocitos de bovino).

Types of supplements	Number of oocytes		inseminated	Number of ova	
	cultured	matured*		fertilized*	cleaved*
ECS	250	80.4±4.7 ^a	250	53.7±4.4 ^a	57.7±3.7 ^a
FCS	250	78.3±4.5 ^a	250	50.4±3.1 ^a	55.4±3.7 ^a
PMSG+hCG	250	85.1±5.1 ^a	250	57.1±3.7 ^a	60.1±4.0 ^c
Control	250	51.9±3.2 ^b	250	32.7±2.9 ^b	20.4±2.0 ^b

^{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.
*(percent)

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 pre-ovulatory 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 (Sanbuiisho 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

- Brackett, B.G. and Z.A. Zuelke. 1993. Analysis of factors involved in the *in vitro* production of bovine embryos. *Theriogenology*, 39: 43-64.
- Fukuda, Y., M. Ichikawa and K. Naito. 1990. Birth of normal calves resulting from bovine oocytes matured, fertilized and cultured with cumulus cells *in vitro* up to the blastocyst stage. *Biology Reproduction*, 42: 114-119.
- Gliedt, D.W., C.F. Rosenkraus, R.W. Rpie, A.L. Munyon, J.N. Pierson, G.F. Miller and J.M. Rakes. 1996. Effects of media, serum, oviductal cells, and hormones during maturation on bovine embryo development *in vitro*. *J. Dairy Sci.*, 79: 536-542.
- Kim, C.I., J.E. Ellington and R.H. Foote. 1990. Maturation, fertilization and development of bovine oocytes *in vitro* using TCM-199 and a simple defined medium with co-culture. *Theriogenology*, 33: 433-439.
- Lambert, R.D., M.A. Sirard and C. Bernard. 1986. *In vitro* fertilization of bovine oocytes matured *in vivo* and collected at laparoscopy. *Theriogenology*, 25: 117-133.
- Mattioli, M., M.L. Bacci and G. Galeati. 1991. Effects of LH and FSH on the maturation of pig oocytes *in vitro*. *Theriogenology*, 36: 95-105.
- Mochizuki, H., Y. Fukui and H. Ono. 1991. Effect of the number of granulosa cells added to culture medium for *in vitro* maturation, fertilization and development of bovine oocytes. *Theriogenology*, 36: 973-985.
- Sanbuiisho, A. and W.R. Threlfall. 1990. The influence of serum and gonadotropins on *in vitro* maturation and fertilization of bovine oocytes. *Theriogenology*, 34: 341-347.
- Schellander, K., F. Fuhrer and B.G. Brackett. 1990. *In vitro* fertilization and cleavage of bovine oocyte matured in medium supplemented with estrous cow serum. *Theriogenology*, 33: 477-485.

Recibido: 6-2-97. Aceptado: 12-11-97.

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