Global Journal of Science Frontier Research: D
Agriculture and Veterinary
Volume 17 Issue 4 Version 1.0 Year 2017
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Efficient Production of Goat and Sheep Embryos Under Similar in Vitro Conditions

By José Carlos Ferreira-Silva, Marcelo Tigre Moura, Pábola Santos Nascimento, Pamela Ramos-Deus, Maiana Silva Chaves, Luis Rennan Sampaio Oliveira & Marcos Antonio Lemos Oliveira

Universidade Federal Rural de Pernambuco

Abstract- In vitro embryo production (IVP) protocols often require species-specific adaptations, in order to obtain embryonic development at high rates. However, these differences in IVP protocols make interspecific comparisons more susceptible to technical factors. The work aimed to compare IVP embryo production in small ruminants (e.g. goats and sheep) using identical in vitro oocyte maturation and fertilization. Oocytes were retrieved, matured, and fertilized under identical media and atmosphere conditions, while embryos were cultured under different conditions. Goat embryos were cultured in KSOM medium under co-culture, while sheep embryos were cultured in SOF medium under low oxygen tension without feeder cells. Cleavage, morula, and blastocyst development were similar for both goat and sheep, suggesting that oocyte maturation and fertilization conditions allowed a similar developmental potential for small ruminant species.

Keywords: fertilization, maturation, Capra hircus, Ovis aries, embryogenesis.

GJSFR-D Classification : FOR Code: 630102

Strictly as per the compliance and regulations of :

© 2017. José Carlos Ferreira-Silva, Marcelo Tigre Moura, Pábola Santos Nascimento, Pamela Ramos -Deus, Maiana Silva Chaves, Luis Rennan Sampaio Oliveira & Marcos Antonio Lemos Oliveira. This is a research/review paper, distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License http://creativecommons.org/licenses/by-nc/3.0/), permitting all non commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Efficient Production of Goat and Sheep Embryos Under Similar in Vitro Conditions

José Carlos Ferreira-Silva [α], Marcelo Tigre Moura [σ], Pábola Santos Nascimento [ρ], Pamela Ramos-Deus [Ѡ], Maiana Silva Chaves[¥], Luis Rennan Sampaio Oliveira[§] & Marcos Antonio Lemos Oliveira [χ]

Abstract - In vitro embryo production (IVP) protocols often require species-specific adaptations, in order to obtain embryonic development at high rates. However, these differences in IVP protocols make interspecific comparisons more susceptible to technical factors. The work aimed to compare IVP embryo production in small ruminants (e.g. goats and sheep) using identical in vitro oocyte maturation and fertilization. Oocytes were retrieved, matured, and fertilized under identical media and atmosphere conditions, while embryos were cultured under different conditions. Goat embryos were cultured in KSOM medium under co-culture, while sheep embryos were cultured in SOF medium under low oxygen tension without feeder cells. Cleavage, morula, and blastocyst development were similar for both goat and sheep, suggesting that oocyte maturation and fertilization conditions allowed a similar developmental potential for small ruminant species. Goat and sheep embryos can be produced at comparable efficiencies under identical oocyte maturation and fertilization conditions, suggesting that an IVP protocol that can be used for both goat and sheep could be achieved in the near future. This IVP protocol for small ruminants could be used to address species-specific embryo metabolism adaptation to in vitro conditions.

Keywords: fertilization, maturation, Capra hircus, Ovis aries, embryogenesis.

I. Introduction

In vitro embryo production (IVP) is an important tool to address early embryonic development in mammalian species (Galli et al., 2014). Moreover, IVP became attractive for commercial applications such as increasing progeny numbers from animals of high genetic merit or transgenic lines (Galli et al., 2014; Ferreira-Silva et al., 2017abc). Species-specific requirements play an important role on IVP commercial potential in livestock, since IVP efficiency remains high in cattle, variable in small ruminants and under development in swine and horses (Galli et al., 2014; Paramio and Izquierdo, 2014; Leemans et al., 2016; Romar et al.; 2016).

Small ruminant species have differences in reproductive physiology, such as estrus duration, moment of ovulation and number of follicular waves (Webb et al., 2004; Tenório Filho et al., 2007; Ferreira-Silva et al., 2017a). Furthermore, recapitulation of early embryonic development required development of IVP protocols that meet these species-specific requirements. Important differences have been observed for in vitro fertilization and embryo culture for these species (Izquierdo et al., 1998; Conceição et al., 2015/2016), while other factors remain to be compared. Moreover, other reports suggested that these differences may be overcome by protocol adaptations, particularly to embryo culture (Paramio and Izquierdo, 2014/2016).

Despite this tendency for independent development of IVP protocols for each species, this fact represents an additional hurdle for interspecific comparisons at gene expression or cellular levels, since embryos are more susceptible to protocol variations. The development of a similar IVP protocol for both goats and sheep could allow addressing relevant processes, such as species-specific susceptibility to embryo in vitro conditions (Mcevoy et al., 2003; Hill 2014). This understanding may aid the development of IVP conditions that diminish pregnancy loss and newborn death known as large offspring syndrome (Mcevoy et al., 2003; Hill, 2014). Moreover, the improvement of IVP systems may also lead to more efficient cryotolerance of such embryos (Katska-Ksiazkiewicz et al., 2004; Rodríguez-Dorta et al., 2007), which remains as a major bottleneck for IVP application in small ruminants.

Thus, using an identical protocol for embryo IVP for several species, species-specific adaptation processes to in vitro conditions may be identified and characterized. The work was aimed to compare IVP embryo production in small ruminants under similar protocol conditions.

II. Materials and Methods

The procedures performed throughout the experiment were formally approved by the Veterinary Medicine Ethics Committee of University Federal Rural of Pernambuco (Protocol 35/2016).

Ovaries were retrieved from mix-breed does and ewes with age varying from 12 to 46 months, which were slaughtered at Suimax, in Igarassu, Pernambuco state, Brazil (latitude 08° 03' 14" S, longitude 34° 52' 52" W). Ovaries were transported in saline solution

Author α σ ρ Ѡ ¥ χ: Laboratorio de Biotécnicas Reprodutivas, Universidade Federal Rural de Pernambuco, Recife-PE, Brazil.
e-mail: carlos.ztec@gmail.com
Author §: Universidade Federal Rural da Amazônia, Parauapebas-PA, Brazil.

© 2017 Global Journals Inc. (US)

supplemented with 30 μg mL^{-1} of gentamicin sulfate at 30 oC to the laboratory within one-hour period after the slaughter.

Cumulus-oocyte complexes (COCs) were retrieved from 2-6 mm follicles by "slicing" as proposed by Bezerra et al. (2014). Follicular fluid was deposited in a petri dish containing collection medium (CM), as described by Conceição et al. (2015).

The selection of COCs was based on their morphology, as recommended by Chiamenti et al. (2013) and Conceição et al. (2015) and further washed three times in medium. Subsequently, pools of 25 oocytes were transferred to 100 μL drops of IVM medium, which was formulated by TCM-199 supplemented with 50 μg mL^{-1} sodium pyruvate, 2.6 mg mL^{-1} sodium bicarbonate, 10% fetal bovine serum (FBS), 50 μg mL^{-1} gentamicin sulfate, 20 μg mL^{-1} FSH/LH (Pluset®) and 1 mg mL^{-1} PVA. COCs were placed in incubator with saturated humidity, 5% de CO2 at 39 °C for 24 hours.

a) In vitro fertilization (IVF)

The IVF was performed using fresh semen, as reported by Conceição et al. (2016). Carefully, 0.1 mL semen was deposited in conical tubes containing 1.5 mL in modified defined medium (mDM) as described by Conceição et al. (2016). Moreover, tubes were placed in a 45o angle position, in order to obtain sperm ascending migration. After 45 minutes of "swim up", the upper 0.8 mL from each tube was aspirated and centrifuged at 350 g for 10 minutes. The supernatant was discarded and 200 μL mDM containing 10 μg mL^{-1} heparin was gently mixed with 200 μL of sperm pellet obtained from centrifugation, as informed by Conceição et al. (2016) and Ferreira-Silva et al. (2017a).

Before IVF, oocytes were scored for cumulus cell expansion and washed in mDM. Moreover, groups of 15 to 20 oocytes were transferred to 100 μL mDM drops containing a sperm suspension of 2 x 106 under paraffin oil. Oocytes and sperm cells were co-incubated under identical to IVM conditions for 18 hours.

b) Embryo culture

Goat presumptive zygotes were cultivated in Potassium simplex optimized medium (KSOM) medium (85.15 mM sodium chloride, 8.0 mM potassium chloride, 2.0 mM calcium chloride, 0.5 mM magnesium chloride, 25 mM sodium bicarbonate, 5 mM sodium lactate, 0.5 mM sodium pyruvate, 2.0 mM glucose, 4.9 mM glycine, 0.1 mM L-glutamine) and supplemented with 10% estrus sheep serum and co-culture with cultured oviduct cells under high oxygen tension of 5% $_{CO}2$ and 20% $_{O}2$ as reported by Conceição et al. (2016) and Ferreira-Silva et al. (2017a).

Sheep presumptive zygotes were denuded by vortexing in modified Synthetic oviduct fluid (mSOF) and pools of 25 presumptive zygotes were transferred to 100 μL drops of mSOF supplemented with 10% FBS under paraffin oil, as described by Bezerra et al. (2014). Embryos were incubated under saturated humidity with 5% $_{CO}2$, 5% $_{O}2$ and 90% N2 at 39 °C. Goat and sheep embryos were cultured for seven days, while embryonic development was assessed for early cleavage at day 3 (D3), late cleavage at day 4 (D4), morula at day 5 (D5) and blastocyst development at day 7 (D7).

c) Statistical Analysis

One-way analysis of variance was performed by minimum squares method by PROC GLM (for fixed variables) and PROC MIXED (for fixed and random variables) on SAS STAT package (SAS institute, Cary, NC). The data was previously analyzed for variance analysis by the Shapiro-Wilk test (variable homogeneity and residual normality). Dependent and independent variables were established based on experimental design. Statistical models considered main effects and all possible interactions. Differences with 5% probability were considered significant.

III. Results

A total of 12 replicates was performed using 1,249 oocytes from both species. In each replicate, COCs were subjected to IVM, IVF and embryo culture. Results for retrieved oocytes, matured oocytes and those subjected to in vitro fertilization are described in Table 1. Mean oocyte retrieval and oocyte survival after IVM and IVF were similar for both species (Table 1). No notable difference was observed in cumulus cell expansion after IVM (data not shown).

Table 1: Retrieved oocytes, in vitro maturation (IVM), and in vitro fertilization (IVF) for goats and sheep under similar conditions.

In vitro Production	Goat (Mean and SD)	Sheep (Mean and SD)
Oocyte retrieval	105.75 ± 6.53	102.58± 4.46
IVM	53.18 ± 6.25	55.16 ± 5.60
IVF	28.41 ± 3.75	29.33 ± 3.96

SD: standard deviation.

Embryonic development rates were determined at days 3, 4, 5, and 7 after IVF, in order to better estimate embryo development kinetics (Table 2). Cleavage rates at days 3 and 4 were similar between species, suggest-ing that oocyte competence and early development al kinetics were indistinguishable (Table 2). Moreover, embryo development at morula and blastocyst stages were also similar for goats and sheep, ruling out any effect of embryo culture condition on development kinetics (Table 2).

© 2017 Global Journals Inc. (US)

Table 2: Cleavage, morula and blastocyst formation for goat and sheep in vitro production under similar conditions.

Development Stage#	Goat (Mean and SD)	Sheep (Mean and SD)
Cleavage(D3)	23.83 ± 1.02	24.91 ± 1.72
Cleavage(D4)	22.25± 1.28	22.58± 1.44
Morula(D5)	20.75 ± 1.28	21.08 ± 1.24
Blastocyst (D7)	6.91 ± 0.79	9.16 ± 1.40

#Days of embryo culture after in vitro fertilization. SD: standard deviation.

IV. Discussion

Goat and sheep oocytes were matured and subject to in vitro fertilization under identical conditions. In vitro embryo production represents an attractive approach to investigate preimplantation development in mammalian species (Galli et al., 2014; Conceição et al., 2015), by allowing to determine minimum culture conditions for embryo development. Furthermore, it represents an alternative source of embryos for biochemical and molecular analysis and as a platform for other approaches such as production of cloned or transgenic animals (Galli et al., 2014; Souza-Fabjan et al., 2014). Several lines of evidence demonstrate that most species require protocol adaptations for adequate embryonic development under in vitro conditions (Chiamenti et al., 2010/2012; Galli et al., 2014; Paramio and Izquierdo, 2014; Ferreira-Silva et al., 2017a).

Oocyte maturation was efficient for both goats and sheep under identical IVM conditions, as described previously in independent studies from our group (Bezerra et al., 2014; Conceição et al., 2015) and for other livestock species (Galli et al., 2014; Paramio and Izquierdo, 2014; Ferreira-Silva et al., 2017a). Although the assay used in the present study is indirect in order to determine IVM efficiency (e.g. cumulus cell expansion), further embryonic development demonstrates that eggs from both species held comparable oocyte competence.

The protocol for IVF in goat and sheep was performed using a combination of mDM medium with heparin. Previous studies in ruminant species have found particularities in their requirement for IVF conditions (Galli et al., 2014; Paramio and Izquierdo, 2014). Goats and cattle require heparin for sperm capacitation (Chiamenti et al., 2010; Galli et al., 2014; García-Álvarez et al., 2015; Ferreira-Silva et al., 2017a), while capacitation factors remain elusive for sheep (García-Álvarez et al., 2015). Due to this fact, IVF in sheep is generally performed under estrus serum-containing media (Paramio and Izquierdo, 2014; García-Álvarez et al., 2015). The results described here suggest that heparin usage for sheep sperm capacitation should be revisited in detail.

Embryo development was similar for both species, despite usage of different embryo culture media and atmosphere conditions. Embryo culture conditions play a major role on embryo quality. Since species also hold differences in their susceptibility to culture conditions (Mcevoy et al., 2003; Hill, 2014), this step on IVP protocol could represent a major hurdle to development of a IVP protocol applicable for ruminant and other livestock species. However, most reports in small ruminants use SOF medium for embryo culture (Paramio and Izquierdo, 2016), suggesting that an identical protocol for IVP in goats and sheep could be achieved in the near future.

The development of an IVP protocol that can be used for both goat and sheep may have important scientific and commercial implications. For research purposes, it further allows the investigation of species-specific embryo metabolism adaptation to in vitro conditions (Mcevoy et al., 2003; Hill, 2014). This investigation may lead to protocols that increase pregnancy rates and newborn viability (Mcevoy et al., 2003; Hill, 2014). Moreover, an improved IVP system for both small ruminant species may increase embryo cryotolerance (Katska-Ksiazkiewicz et al., 2004; Rodríguez-Dorta et al., 2007). This increased cryotolerance may be particularly achieved if embryo metabolism under improved IVP conditions leads to reduced lipid accumulation in embryonic cells (Romão et al., 2015).

This protocol could also ease media production for commercial small ruminant laboratories. In order to achieve such goals, appropriate embryo culture conditions for both goat and sheep embryos need to be identified using oocyte maturation and fertilization described above.

V. Conclusion

Goat and sheep embryos can be produced at comparable efficiencies under identical oocyte maturation and fertilization conditions, suggesting that a IVP protocol useful for both goat and sheep could be achieved in the near future.

a) Conflict of interest

The authors declare no conflict of interest.

b) Acknowledgements

This study was funded by "National Counsel of Technological and Scientific Development" (CNPq) and Coordination for the Improvement of Higher Education Personnel (CAPES) grants.

c) Author Contribution

J.C.F.S., M.T.M., M.A.L.O. conceived the research; J.C.F.S., M.T.M., P.S.N., P.R.D., M.S.C., L.R.S.O. performed the research. J.C.F.S., M.T.M., M.A.L.O. analyzed the data and wrote the manuscript.

© 2017 Global Journals Inc. (US)

References Références Referencias

1. Bezerra, F. Q. G., Silva, J. C. F., Silva, P. G. C., Cantanhêde, L. F., Basto, S. R. L., Neto, L. F., ... & Oliveira, M. A. L. (2014). Produção in vitro de embriões ovinos a partir de oócitos coletados durante os períodos seco e chuvoso. Medicina Veterinária (UFRPE), 8(3), 17-23.
2. Chiamenti, A., Aguiar Filho, C. R., Freitas Neto, L. M., Chaves, R. M., Paula-Lopes, F. F., Lima, P. F., ... & Oliveira, M. A. L. (2010). Effects of retinoids on the in vitro development of Capra hircus embryos to blastocysts in two different culture systems. Reproduction In Domestic Animals, 45(5).
3. Chiamenti, A., Filho, C., Moura, M., Paula-Lopes, F., Neves, J., Neto, C., ... & Oliveira, M. (2012). Use of retinyl acetate, retinoic acid and insulin-like growth factor-I (IGF-I) to enhance goat embryo production. Acta Veterinaria Hungarica, 61(1), 116-124.
4. Conceição, J. C., Moura, M. T., Ferreira-Silva, J. C., Cantanhêde, L. F., Chaves, R. M., Lima, P. F., & Oliveira, M. A. (2016). Incidence of apoptosis after retinoids and insulin-like growth factor-I (IGF-I) supplementation during goat in vitro embryo production. Zygote, 24(6), 808-813.
5. Conceição, J. C., Moura, M. T., Ferreira-Silva, J. C., Ramos-Deus, P., Silva, P. G., Cantanhêde, L. F., ... & Oliveira, M. A. (2015). Use of retinoids during oocyte maturation diminishes apoptosis in caprine embryos. Acta Veterinaria Hungarica, 63(2), 234-242.
6. Ferreira-Silva, J. C., Basto, S. R. L., Tenório Filho, F., Moura, M. T., Silva Filho, M. L., & Oliveira, M. A. L. (2017a). Reproductive performance of postpartum ewes treated with insulin or progesterone hormones in association with ram effect. ReproductionIn Domestic Animals, 52, 1-7.
7. Ferreira-Silva, J. C., Burnett, T. A., Souto, P. F. M. P., Pereira, L. C., Araujo, M. V., Moura, M. T., & Oliveira, M. A. L. (2017b). Progesterone (P4), luteinizing hormone (LH) levels and ovarian activity in postpartum Santa Inês ewes subject to a male effect. Archives Animal Breeding, 60(2), 95-100.
8. Ferreira-Silva, J. C., Moura, M. T., Silva, T. D., Oliveira, L. R. S., Chiamenti, A., Freitas, V. J. F., & Oliveira, M. A. L. (2017c). Full-term potential of goat in vitro produced embryos after different cryopreservation methods. Cryobiology, 75, 75-79.
9. Galli, C., Duchi, R., Colleoni, S., Lagutina, I., & Lazzari, G. (2014). Ovum pick up, intracytoplasmic sperm injection and somatic cell nuclear transfer in cattle, buffalo and horses: from the research laboratory to clinical practice. Theriogenology, 81(1), 138-151.
10. García-Álvarez, O., Maroto-Morales, A., Jiménez-Rabadán, P., Ramón, M., Del Olmo, E., Iniesta-Cuerda, M., ... & Soler, A. J. (2015). Effect of different media additives on capacitation of frozen–thawed ram spermatozoa as a potential replacement for estrous sheep serum. Theriogenology, 84(6), 948-955.
11. Hill, J. R. (2014). Incidence of abnormal offspring from cloning and other assisted reproductive technologies. Annu. Rev. Anim. Biosci., 2(1), 307-321.
12. Izquierdo, D., Villamediana, P., Palomo, M. J., Mogas, T., & Paramio, M. T. (1998). Effect of sperm capacitation and fertilization media on IVF and early embryo development of prepubertal goat oocytes. Theriogenology, 49(8), 1501-1513.
13. Kątska-Książkiewicz, L., Ryńska, B., Gajda, B., & Smorąg, Z. (2004). Effect of donor stimulation, frozen semen and heparin treatment on the efficiency of in vitro embryo production in goats. Theriogenology, 62(3), 576-586.
14. Leemans, B., Gadella, B. M., Stout, T. A., De Schauwer, C., Nelis, H., Hoogewijs, M., & Van Soom, A. (2016). Why doesn't conventional IVF work in the horse? The equine oviduct as a microenvironment for capacitation/fertilization. Reproduction, 152(6), R233-R245.
15. McEvoy, T. G., Ashworth, C. J., Rooke, J. A., & Sinclair, K. D. (2003). Consequences of manipulating gametes and embryos of ruminant species. Reproduction(Cambridge) 61, 167-182.
16. Olivares, C. C. S., de Souza-Fabjan, J. M. G., da Fonseca, J. F., Balaro, M. F. A., de Figueirêdo Freitas, V. J., de Oliveira, R. V., & Brandão, F. Z. (2017). Comparison of different sperm selection techniques in ram frozen-thawed sperm. Acta Scientiae Veterinariae, 45, 1-11.
17. Paramio, M. T., & Izquierdo, D. (2014). Current status of in vitro embryo production in sheep and goats. Reproduction In Domestic Animals, 49(s4), 37-48.
18. Paramio, M. T., & Izquierdo, D. (2016). Recent advances in in vitro embryo production in small ruminants. Theriogenology, 86(1), 152-159.
19. Rodriguez-Dorta, N., Cognié, Y., Gonzalez, F., Poulin, N., Guignot, F., Touzé, J. L., ... & Gracia, A. (2007). Effect of coculture with oviduct epithelial cells on viability after transfer of vitrified in vitro produced goat embryos. Theriogenology, 68(6), 908-913.
20. Romão, R., Marques, C. C., Baptista, M. C., Barbas, J. P., Horta, A. E. M., Carolino, N., ... & Pereira, R. M. (2015). Cryopreservation of in vitro–produced sheep embryos: Effects of different protocols of lipid reduction. Theriogenology, 84(1), 118-126.
21. Romar, R., Funahashi, H., & Coy, P. (2016). In vitro fertilization in pigs: New molecules and protocols to consider in the forthcoming years. Theriogenology, 85(1), 125-134.

© 2017 Global Journals Inc. (US)

22. Souza-Fabjan, J. M. G., Panneau, B., Duffard, N., Locatelli, Y., De Figueiredo, J. R., de Figueirêdo Freitas, V. J., & Mermillod, P. (2014). In vitro production of small ruminant embryos: Late improvements and further research. Theriogenology, 81(9), 1149-1162.
23. Tenório Filho, F., Santos, M. H. B., Carrazzoni, P. G., Paula-Lopes, F. F., Neves, J. P., Bartolomeu, C. C., ... & Oliveira, M. A. L. (2007). Follicular dynamics in Anglo-Nubian goats using transrectal and transvaginal ultrasound. Small Ruminant Research, 72(1), 51-56.
24. Webb, R. P. C. G., Garnsworthy, P. C., Gong, J. G., & Armstrong, D. G. (2004). Control of follicular growth: local interactions and nutritional influences. Journal of Animal Science, 82(13_suppl), e63-e74.

© 2017 Global Journals Inc. (US)

This page is intentionally left blank

© 2017 Global Journals Inc. (US)