Theriogenology 85 (2016) 1262–1266

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Serum profile of cytokines interferon gamma and interleukin-10 in ewes subjected to artificial insemination by cervical retraction

C.T.G. Alvares [a], J.F. Cruz [b,*], C.C. Romano [c], F.Z. Brandão [d]

[a] Estação Experimental Fazenda Almada, Departamento de Ciências Agrárias e Ambientais, Universidade Estadual de Santa Cruz, Ilhéus, Bahia, Brazil
[b] Laboratório de Reprodução de Caprinos e Ovinos, Departamento de Fitotecnia e Zootecnia, Universidade Estadual do Sudoeste da Bahia, Vitória da Conquista, Bahia, Brazil
[c] Centro de Biotecnologia e Genômica, Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Ilhéus, Bahia, Brazil
[d] Laboratório de Reprodução Animal, Faculdade de Veterinária, Universidade Federal Fluminense, Niterói, Rio de Janeiro, Brazil

ARTICLE INFO

Article history:
Received 28 May 2015
Received in revised form 16 December 2015
Accepted 16 December 2015

Keywords:
Embryo loss
Implantation
Inflammation
Sheep
T-helper 1/T-helper 2
Transcervical insemination

ABSTRACT

This study evaluated the influence of artificial insemination (AI) by cervical retraction (CRI) on serum levels of interferon gamma (IFNγ) and interleukin-10 (IL-10) in ewes. Synchronized pluriparous Santa Inês ewes were subjected to natural mating (NM, n = 8) and AI, which was performed for a fixed time (55 ± 1 hour) by CRI (n = 8) or laparoscopy (n = 8). Ewes were classified as pregnant, with return to estrus (RE) or with embryonic loss (EL). Blood samples were collected on Day 0, Day 3, Day 5, Day 12, and Day 17 (Day 0 = AI/NM) for progesterone dosage and cytokines were quantified from Day 0 to Day 12. Progesterone levels were constant, except for a decrease in ewes with RE at Day 17 ($P < 0.05$). Regardless of the reproductive method used, there was no difference in the IFNγ and IL-10 levels at any time, with averages of 642.1, 713.2, and 741.2 pg/mL for IFNγ and 667.1, 616.8, and 721.1 pg/mL for IL-10 when using CRI, laproscopy, and NM, respectively. Regarding the physiological status, ewes with EL had lower serum levels of IFNγ and IL-10 than pregnant ewes and ewes with RE, regardless of the reproductive method used, with averages of 769.1, 714.9, and 555.7 pg/mL for IFNγ and 713.8, 699.3, and 578.7 pg/mL for IL-10 in pregnant ewes, ewes with RE and EL, respectively ($P < 0.01$). In conclusion, AI by CRI in Santa Inês ewes does not alter the profile of serum cytokines IFNγ and IL-10 and does not induce an inflammatory reaction that can compromise pregnancy.

1. Introduction

Intrauterine artificial insemination (AI) in sheep with the use of frozen semen still faces obstacles. Despite laparoscopy giving the best fertility results, it is not widely used because of its high costs and need for anesthetic procedures and specialized personnel [1]. To circumvent these limitations, transcervical AI technique was developed, which enables the intrauterine deposition of semen through the cervical canal [2].

However, transcervical AI has yet to become a widely used technique, owing mostly to the sinuousness of the sheep cervix and its variable fertility success rate [3,4]. Several alternatives have been created to improve the performance of transcervical AI, including the use of cervix-dilating drugs [5–7] and development of tools adapted to the cervical anatomy [8–10].

Some studies suggest that cervical handling may cause tissue damage, thereby altering the uterine environment by proinflammatory cells, which could subsequently result in loss of fertility or embryonic death [11–13]. The immune system plays a decisive role in reproduction, because it protects the organism against external pathogens and

* Corresponding author. Tel.: +55 77 3424 8627; fax: +55 77 3424 1059.
E-mail address: jurandirferreirac@gmail.com (J.F. Cruz).

0093-691X/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2015.12.009

modulates the process that avoids rejection of a "semi-allograft" conceptus [14].

Cytokines are a heterogeneous and pleiotropic group of hydrosoluble extracellular polypeptides or glycoproteins with molecular weights ranging from 5 to 100 kDa. They are chemical signals that trigger biochemical cascades, resulting in the production of other cytokines, and ultimately regulating the inflammatory response [15].

T-helper 1 (Th1) cytokines are considered as proinflammatory and T-helper 2 (Th2) cytokines as anti-inflammatory. In the reproductive system, both contribute to the communication among cells, and they are not only secreted by embryos but also by peripheral blood lymphocytes, macrophages, endometrial cells, and cells from the uterine tubes [16].

Interferon gamma (IFNγ) is one of the main cytokines secreted by Th1 cells. Activated monocytes secrete proinflammatory interleukins (ILs) IL-12, IL-15, and IL-18, which enhance the cytotoxic activity of natural killer (NK) cells and CD4 + CD8 + T cells. Consequently, paracrine and systemic IFNγ secretion activate cytolytic and cytotoxic immunity [17,18]. On the other hand, IL-10 is an important Th2 cytokine secreted by several immune and nonimmune cells, which induces the production of antibodies through B cells and regulates the release of Th1 cytokines. Thereby, playing immunosuppressant and tolerogenic roles [19,20].

Numerous studies indicate that some extent of systemic or uterine inflammation is necessary for the normal process of embryo implantation, which demands a Th1/Th2 balance that can successfully control the maternal immune system response against conceptus tissues [21,22]. Tissue or systemic alterations that markedly disturb that Th1/Th2 balance may cause reproductive disorders such as infertility, embryonic loss (EL), and abortion [23,24].

Considering the inconsistency in fertility rates of transcervical AI, compared with those of laparoscopy [3,13], we hypothesized that cervical manipulation can lead to inflammatory reaction capable of raising serum Th1/Th2 ratio, thereby threatening the viability of the embryo. Thus, this study aimed to evaluate the influence of AI by cervical retraction on serum IFNγ and IL-10 levels in Santa Inês ewes.

2. Materials and methods

2.1. Animals and treatments

The experimental procedures performed in this study were approved by the Ethics Committee in the Use of Animals of the Southwest Bahia State University, under protocol no. 68/2014.

The study was conducted in the sheep farming station of the Santa Cruz State University, Ilhéus, Bahia, (14°47′20″S, 39°02′56″W). Twenty-four Santa Inês pluriparous ewes from commercial herds (4–8 years, weighing 52.20 ± 4.60 kg, with a body condition score 3.4 ± 0.4, scale 0–5 [25]) were used. One ram and four teaser males were also used. Animals grazed in pastures of *Brachiaria humidicola* from 8 AM to 4 PM and were supplemented with *Pennisetum purpureum* and corn/soy-based concentrates (15% crude protein) at a rate of 400 g/animal/day. Vitamin-mineral premix (Guabiphos Ovinos AE; Guabi, Brazil) and water were available ad libitum. Hormonal protocol consisted of intravaginal sponges containing 60 mg of medroxyprogesterone acetate (Progespon; Zoetis, Brazil), which were kept in place for 12 days. After sponge withdrawal, 200 IU i.m. of eCG (Folligon, MSD Saúde Animal, Brazil) was administered. Ewes were bred using transcervical AI by cervical retraction (CRI, n = 8), AI by laparoscopy (LAI, n = 8), or natural mating (NM, n = 8). AI was conducted 55 ± 1 hour after eCG administration. In the NM group, ewes were mated in the subsequent natural estrus to the hormonal protocol.

For the CRI, the ewes were kept standing in a cradle (standing position). After the vulvar region was sanitized, a 15-cm vaginal speculum with a light source was used to visualize the cervical os, and the cervix was retracted out to the vulvar opening. Fixation was achieved using two 25-cm Allis tweezers. Next, a 12-cm metal applicator containing a mandrel (Aplicador Expansor Ovino; Alta Genetics, Brazil) was used to cross the cervical rings as much as possible; then, a 0.25-mL straw was introduced, containing the commercially available frozen-thawed semen.

The LAI procedure was adapted from Evans and Maxwell [26]. The ewes were previously submitted to a 24-hour fasting period. Afterward, their abdominal regions were shaved and disinfected, and the ewes were placed in a special cradle in dorsal recumbency at an angle of 60°, with their hind feet raised. Local anesthesia was performed by injecting lidocaine hydrocloride 2% (Anestésico L; Pearson, Brazil) at two abdominal points near the uterine horns. After making two small incisions, two trocars were inserted: one to introduce the laparoscope (Karl Storz, Germany), and the other one for multiple uses: pumping air, using a handler to locate the uterus, or introducing the insemination pipette with a puncturing end. Once the uterine horns were located, the insemination was performed with the frozen-thawed semen, using half of the dose (0.25 mL) for each uterine horn.

2.2. Progesterone dosage

To establish serum progesterone (P_4) concentration, blood samples were collected from the ewes on Day 0, Day 3 Day 5, Day 12, and Day 17. Day 0 corresponds to the day when the AI or NM was performed. The samples were collected from the jugular veins using needles and vacuum blood collection tubes with no anticoagulant (BD Vacutainer; England). The samples were centrifuged at 1500 × *g* for 10 minutes and the obtained serum was stored in a freezer at −20 C. Hormone dosing was performed with duplicate samples through a radioimmunoassay in the Animal Reproduction Laboratory of the Federal Fluminense University, Rio de Janeiro. In order to quantify P_4, a progesterone radioimmunoassay kit (IM1188, Beckman Coulter; Immunotech, Czech Republic) was used, with a sensitivity of 0.05 ng/mL and with intraassay and interassay coefficients of variation of 6.5% and 7.2%, respectively.

2.3. Reproductive rates and physiological status

Non-return to estrus (RE) was determined with the aid of teasers up to 21 days after AI or NM. The pregnancy diagnosis was conducted through ultrasonography

(Falco Vet 100, Pie Medical, Netherlands), with a rectal transducer at 8 MHz, at 35 days after AI or NM. The ewes were characterized as pregnant, with RE or with EL considering estrous cycle analysis, serum progesterone concentration and ultrasonographic diagnosis.

2.4. IFNγ and IL-10 dosage

Duplicate serum samples from Day 0, Day 3, Day 5, and Day 12 were used to detect cytokines IFNγ and IL-10. Using specific ovine commercial ELISA kits (Neobiolab, USA), 96 well polystyrene plates were previously sensitized with anticytokine antibodies. Control cytokine samples were used at concentrations of 1000 pg/mL and 50 pg/mL to establish the standard curve 100 μL serum samples, which had been previously diluted in buffer by 1:10, and 100 μL standard concentration samples were added to a microplate, simultaneously with 30 μL of a conjugate of the corresponding cytokine with peroxidase. After being incubated in a humid chamber at 37 C for 1 hour, the plate was washed five times in an automated washer (Termo Scientific, USA) with 0.05% PBS Tween. After adding 50 μL of substrate solution (tetramethylbenzidine), the microplate was incubated in the dark at room temperature for 15 minutes. The reaction was interrupted with sulfuric acid 1M. The spectrophotometric reading was performed in the microplate reader (Thermoplate) at a wavelength of 450 nm.

2.5. Statistical analysis

Subjects were grouped according to the reproductive method (AI by CRI, LAI, or NM), or pregnancy diagnosis (pregnant, with RE, or having undergone EL). The progesterone concentration data were analyzed and the means were compared using the Student-Newman-Keuls method (PROC GLM, SAS, version 9.1). Differences were considered significant when $P < 0.05$. The data regarding serum levels of cytokines (IFNγ and IL-10) were analyzed and the means were compared using the Tukey's test (GraphPad Prism, version 6; GraphPad Software, USA). Differences were considered significant when $P < 0.01$.

3. Results

The rate of RE was 25% in the NM group and 37.5% in both CRI and LAI groups. The pregnancy rate was 62.5% in NM ewes and 37.5% inseminated ewes (CRI or LAI). Total ELs were 12.5% in the NM group and 25% in the CRI and LAI groups.

The serum levels of P_4 were similar until Day 12 in pregnant ewes, ewes with RE and EL, with mean values of 0.75, 1.14, 2.28, and 5.01 ng/mL on Day 0, Day 3, Day 5, and Day 12, respectively. On Day 17, P_4 levels in pregnant ewes and ewes with EL remained high (5.98 and 5.32 ng/mL) and were higher than those in ewes with RE ($P < 0.05$), in which a marked reduction to 0.63 ng/mL was observed (Fig. 1).

Throughout the following estrous cycle (Day 0, Day 3, Day 5, and Day 12), the serum levels of cytokines were similar for the different reproductive methods, with means of 642.1, 713.2, and 741.2 pg/mL for IFNγ and 667.1, 616.8, and 721.1 pg/mL for IL-10 in ewes that were submitted to

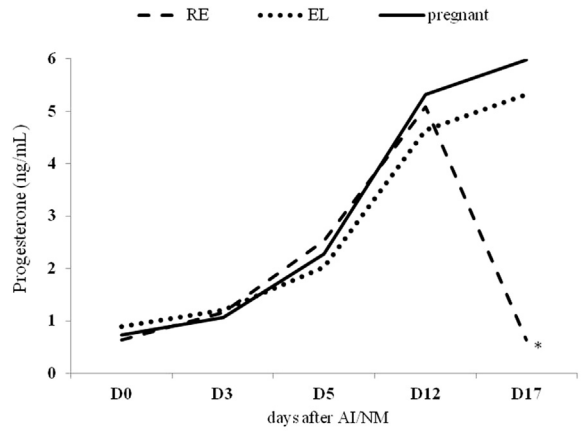

Fig. 1. Serum progesterone (mean) of pregnant (n = 11) Santa Inês ewes, with EL (n = 5) or RE (n = 8), regardless of the reproductive method used (AI: artificial insemination; NM: natural mating). *Mean with superscript differs ($P < 0.05$) from others within this same day. AI, artificial insemination; EL, embryo loss; NM, natural mating; RE, return to estrus.

CRI, LAI, or NM, respectively, although a greater variation was observed within the CRI group, with minimum and maximum values of 44.0 and 890.0 pg/mL, respectively, when compared to the LAI (220.0 and 907.0 pg/mL) and NM (368.0 and 860.0 pg/mL) groups.

Regarding the physiological status, the serum cytokine levels were shown to be lower in ewes that underwent EL than in pregnant and ewes with RE ($P < 0.01$), regardless of the type of insemination. IFNγ levels were 769.1, 714.9, and 555.7 pg/mL, and IL-10 levels were 713.8, 699.3, and 578.7 pg/mL for pregnant, ewes with RE, and ewes having undergone EL, respectively (Fig. 2). The IFNγ/IL-10 ratio remained at 1.07, 1.02, and 0.96, in that same order.

4. Discussion

The proportion of pregnant ewes to those with RE was higher in the NM group than in the CRI and LAI groups, as expected, because frozen-thawed semen has less fertilization ability [27]. The number of ewes with EL is in agreement with other reports, ranging from 5% to 30% [28,29].

The P_4 secretion curve followed the expected physiological pattern, with no influence from the reproductive methods used. The occurrence of luteolysis was clear in ewes with RE from the marked reduction of P_4 levels between Day 12 and Day 17, unlike what was observed for pregnant ewes. This RE may be associated either with failed fertilization, because of possible functional damage or impairment of transport of spermatozoa, or with abnormalities in embryonic development, owing to complications attributable to the aging of gametes [27].

According to Bazer et al. [30], luteolysis in ewes with RE is expected between days 15 and 16 of the estrous cycle, due to the endometrial secretion of PGF2α. On the other hand, the presence of a conceptus generates antiluteolytic signaling through the secretion of interferon tau (IFNτ), providing hormonal recognition of pregnancy. In the case of ewes that underwent EL, the high P_4 level up to Day 17

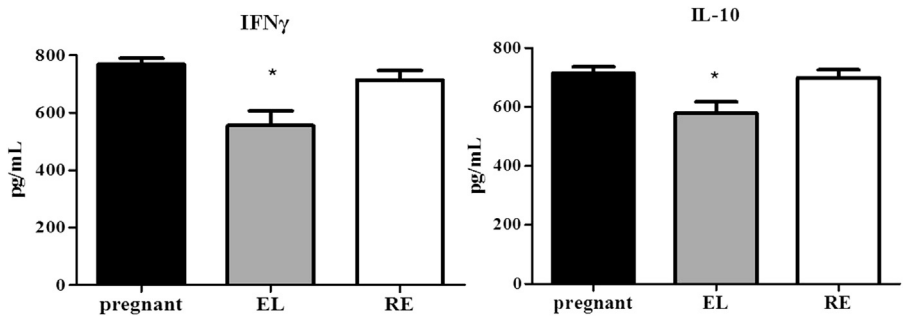

Fig. 2. Serum IFNγ and IL-10 (means ± SEM) of pregnant (n = 11) Santa Inês ewes, with EL (n = 5) or with RE (n = 8), regardless of the reproductive method used (AI by cervical retraction, laparoscopy, or natural mating). *Mean with superscript differs (P < 0.01) from others. EL, embryo loss; RE, return to estrus.

suggests the presence of a viable embryo, which is capable of secreting IFNτ and preventing luteolysis [16]. The combination of P_4 and IFNτ favors the activation of interferon-stimulated genes, which are involved in tasks such as the transportation of antiviral compounds and the protection of conceptus tissues against maternal immune cells [21].

The serum IFNγ levels were similar among the reproductive methods. The estrus cycle is physiologically characterized by a local inflammatory cascade toward cervical dilation [31]. Therefore, handling the cervix during CRI, with possible tissue damage, could intensify this inflammation [12], leading to higher monocyte activation, proinflammatory (IL-12) cytokine secretion, and the consequent release of IFNγ NK cells and T lymphocytes [32,33]. However, in this study the serum levels of IFNγ in ewes subjected to CRI remained regular and similar to those found in ewes subjected to LAI or NM, and no traces of exacerbation of inflammatory reaction due to the CRI procedure were observed.

The similarity in serum levels of IL-10 in all reproductive methods suggests that, even if inflammatory reactions have taken place due to CRI, they would not have been sufficient to trigger a Th2 response, because this situation is an action that regulates the Th1 response [34]. In cases of higher proinflammatory Th1 activity, the systemic IFNγ/IL-10 ratio is also high, which also impairs the viability of pregnancies [35]. Therefore, the no influence of CRI is reinforced for the similar IFNγ/IL-10 ratio in the ewes inseminated with the three methods (0.96, 1.15, and 1.03 for CRI, LAI and NM, respectively).

Some investigators suggest that the handling and retraction of the cervix during AI may raise PGF2α production, causing the invasion of the luteal tissue by neutrophils, which results in increased secretion of proinflammatory cytokines—among which IFNγ, and the consequent luteolysis and embryonic death [13,36].

The regularity of serum levels of IFNγ and IL-10 in the 12 days after the AI, in addition to the similar systemic profiles of those cytokines, especially when compared to NM, shows that CRI performed in standing position and with low stress intensity does not induce inflammatory reactions capable of compromising late embryo viability.

The main IFNγ-secreting sources are NK cells, which aim to provide an antiviral activity and an immune cell response against pathogens [17]. However, studies indicate a central role of IFNγ at the implantation site of embryos, which involves the remodeling of decidual spiral arteries [37]. Lack of IFNγ, which is caused by insufficient NK cells, results in abnormal remodeling, such as the thickening of decidual vessels and reduced vascular lumen [38,39].

Regardless of the reproductive method used, all ewes with IFNγ serum levels under 600 pg/mL have undergone EL, suggesting that levels of IFNγ under that level are insufficient to provide a proper decidual environment, making impossible the pregnancy after Day 17.

In the case of IL-10, the smallest levels observed in ewes that underwent EL point out that those animals may have a less efficient tolerogenic activity than the remaining ewes, especially the pregnant ones. IL-10 plays a crucial role in pregnancy because of its immunosuppressant and regulatory activities with other intrauterine modulators [40]. The lack of IL-10 may cause infertility, defective embryo implantation, and recurring abortions in murines [40,41] and in women [34,42].

On the other hand, studies involving infertility show that the IFNγ/IL-10 ratio tends to increase through the action of Th1 immunity [35,43]. However, the IFNγ/IL-10 ratio was shown to be balanced and similar in the three groups of reproductive methods, despite the serum levels of these cytokines were lower in ewes with EL. This fact suggests that ewes, although apparently in physiological conditions, did not have immune systems compatible with sustaining the pregnancies.

The transcervical AI results may be influenced by the sheep breed [28,44]; therefore, more studies on CRI in other breeds besides Santa Inês are needed before this method can be applied widely.

4.1. Conclusions

Artificial insemination by cervical retraction in Santa Inês ewes performed in standing position does not alter the serum profiles of cytokines IFNγ and IL-10. Therefore, it does not induce an inflammatory reaction capable of compromising pregnancy.

Acknowledgments

The authors thank the Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB), by the financial support under grant APP 0069/2011; Alta Genetics, to donation of transcervical AI catheters (grant no. MAP 20/2011); Laboratório

Sinapse Biogenética, to donation of the kits for ELISA (grant no. 041597/2015).

Competing Interests

The authors declare no conflicts of interest.

References

[1] Windsor DP. Factors influencing the success of transcervical insemination in Merino ewes. Theriogenology 1995;43:1009–18.
[2] Halbert GW, Dobson H, Walton JS, Buckrell BC. A technique for transcervical intrauterine insemination of ewes. Theriogenology 1990;33:993–1010.
[3] Sayre BL, Lewis GS. Fertility and ovum fertilization rate after laparoscopic or transcervical intrauterine artificial insemination of oxytocin-treated ewes. Theriogenology 1997;48:267–75.
[4] Kershaw CM, Khalid M, McGowan MR, Ingram K, Leethongdee S, Wax G, et al. The anatomy of sheep cervix and its influence on the transcervical passage of an inseminating pipette into the uterine lumen. Theriogenology 2005;64:1225–35.
[5] Sayre BL, Lewis GS. Cervical dilation with exogenous oxytocin does not affect sperm movement into the oviducts in ewes. Theriogenology 1996;45:1523–33.
[6] Leethongdee S, Khalid M, Bhatti A, Ponglowhapan S, Kershaw CM, Scaramuzzi RJ. The effects of prostaglandin E analogue misoprostol and follicle-stimulating hormone on cervical penetrability in ewes during the peri-ovulatory period. Theriogenology 2007;67:767–77.
[7] Gündüz MC, Turna Ö, Cirit Ü, Uçmak M, Tek Ç, Sabuncu A, et al. Lambing rate and litter size following carazolol administration prior to insemination in Kivircik ewes. Anim Reprod Sci 2010;118:32–6.
[8] Aral F, Yavuzer Ü, Zonturlu AK. The effect of air pressure with cervical artificial insemination on the fertility of Awassi ewes synchronized with PGF2α. Kafkas Univ Vet Fak Derg 2010;16:37–41.
[9] Álvarez M, Chamorro CA, Kaabi M, Anel-López L, Boixo JC, Anel E, et al. Design and "in vivo" evaluation of two adapted catheters for intrauterine transcervical insemination in sheep. Anim Reprod Sci 2012;131:153–9.
[10] Wulster-Radcliffe MC, Lewis GS. Development of a new transcervical artificial insemination method for sheep: effects of a new transcervical artificial insemination catheter and traversing the cervix on semen quality and fertility. Theriogenology 2002;58:1361–71.
[11] Hawk HW. Sperm survival and transport in the female reproductive tract. J Dairy Sci 1983;66:2645–60.
[12] Campbell JW, Harvey TG, McDonald MF, Sparksman RI. Transcervical insemination in sheep: an anatomical and histological evaluation. Theriogenology 1996;45:1535–44.
[13] Wulster-Radcliffe MC, Wang S, Lewis GS. Transcervical artificial insemination in sheep: effects of a new transcervical artificial insemination instrument and traversing the cervix on pregnancy and lambing rates. Theriogenology 2004;62:990–1002.
[14] Shechter R, London A, Schwartz M. Orchestrated leukocyte recruitment to immune-privileged sites: absolute barriers versus educacional gates. Nat Rev Immunol 2013;13:206–18.
[15] Rice A, Chard T. Cytokines in implantation. Cytokine Growth Factor Rev 1998;9:176–85.
[16] Schäfer-Somi S. Cytokines during early pregnancy of mammals: a review. Anim Reprod Sci 2003;75:73–94.
[17] Pólgar K, Yacono PW, Golan DE, Hill JA. Immune interferon gamma inhibits translational mobility of a plasma membrane protein in preimplantation stage mouse embryos: a T-helper 1 mechanism for immunologic reproductive failure. Am J Obstet Gynecol 1996;174:282–7.
[18] Dorman SE, Holland SM. Interferon-γ and interleukin-12 pathway defects and human disease. Cytokine Growth Factor Rev 2000;11:321–33.
[19] Kalkunte S, Nevers T, Norris WE, Sharma S. Vascular IL-10: a protective role in preeclampsia. J Reprod Immunol 2011;88:165–9.
[20] Fan DX, Duan J, Li MQ, Xu B, Li DJ, Jin LP. The decidual gamma-delta T cells up-regulate the biological functions of trophoblasts via IL-10 secretion in early human pregnancy. Clin Immunol 2011;141:284–92.
[21] Bazer FW, Spencer TE, Johnson GA, Burghardt RC. Wu, G. Comparative aspects of implantation. Reproduction 2009;138:195–209.
[22] Lin Y, Nakashima A, Shima T, Zhou X, Saito S. Toll-like receptor signaling in uterine natural killer cells – role in embryonic loss. J Reprod Immunol 2009;83:95–100.
[23] Krakowski L, Krawczyk CH, Wrona Z, Dąbrowski R, Jarosz L. Levels of selected T lymphocyte subpopulations in peripheral blood of mares which experienced early embryonic death. Anim Reprod Sci 2010;120:71–7.
[24] Makrigiannakis A, Petsas G, Toth B, Relakis K, Jeschke U. Recent advances in understanding immunology of reproductive failure. J Reprod Immunol 2011;90:96–104.
[25] Russel AJF, Doney JM, Gun RG. Subjective assessment of body fat in live sheep. J Agric Sci 1969;72:451–4.
[26] Evans G, Maxwell WMC. Intrauterine insemination. Salamon's artificial insemination of sheep and goats. Sidney: Butterworths; 1987. p. 154–9.
[27] Salamon S, Maxwell WMC. Storage of ram semen. Anim Reprod Sci 2000;62:77–111.
[28] Fair S, Hanrahan JP, O'Meara CM, Duffy P, Rizos D, Wade, et al. Differences between Belclare and Suffolk ewes in fertilization rate, embryo quality and accessory sperm number after cervical or laparoscopy artificial insemination. Theriogenology 2005;63:1995–2005.
[29] Olivera-Muzante J, Fierro S, López V, Gil J. Comparison of prostaglandin and progesterone-based protocols for timed artificial insemination in sheep. Theriogenology 2011;75:1232–8.
[30] Bazer FW, Burghardt RC, Johnson GA, Spencer TE, Wu G. Interferons and progesterone for establishment and maintenance of pregnancy: interactions among novel cell signaling pathways. Reprod Biol 2008;8:179–211.
[31] Mitchell SE, Robinson JJ, King ME, McKelvey WAC, Williams LM. Interleukin 8 in the cervix of non-pregnant ewes. Reproduction 2002;124:409–16.
[32] Bataille NT, Dubanchet S, Coulomb-L'Hermine A, Durand-Gasselin I, Frydman R, Chaouat G. A new role for natural killer cells, interleukin (IL)-12, and IL-18 in repeated implantation failure after in vitro fertilization. Fertil Steril 2004;81:59–65.
[33] Yu J, Wei M, Becknell B, Trotta R, Liu S, Boyd Z, et al. Pro- and anti-inflammatory cytokine signaling: reciprocal antagonism regulates interferon-gamma production by human natural killer cells. Immunity 2006;24:575–90.
[34] Ginsburg ES, Xiao L, Gargiulo AR, Kung FT, Politch JA, Schust DJ, et al. T-helper 2 and 3 type immunity to trophoblast in successful in vitro fertilization – embryo transfer. Fertil Steril 2005;83:1659–64.
[35] Calleja-Agius J, Muttukrishna S, Pizzey AR, Jauniaux E. Pro- and anti-inflammatory cytokines in threatened miscarriages. Am J Obstet Gynecol 2011;205:8–16.
[36] Weems CW, Weems YS, Randel RD. Prostaglandins and reproduction in female farm animals. Vet J 2006;171:206–28.
[37] Ashkar AA, Croy BA. Functions of uterine natural killer cells are mediated by interferon gamma production during murine pregnancy. Semin Immunol 2001;13:235–41.
[38] Guimond BMJ, Wang B, Croy BA. Engraftment of bone marrow from severe combined immunodeficient (SCID) mice reverses the reproductive deficits in natural killer cell-deficient tgε26 mice. J Exp Med 1998;187:217–23.
[39] Lash GE, Bulmer JN. Do uterine natural killer (uNK) cells contribute to female reproductive disorders? J Reprod Immunol 2011;88:156–64.
[40] Murphy SP, Fast LD, Hanna NN, Sharma S. Uterine NK cells mediate inflammation-induced fetal demise in IL10-null mice. J Immunol 2005;175:4084–90.
[41] Christiansen OB, Nielsen HS, Kolte AM. Inflammation and miscarriage. Semin Fetal Neonatal Med 2006;11:302–8.
[42] Ozkan ZS, Deveci D, Kumbak B, Simsek M, Ilhan F, Sekersioglu S, et al. What is the impact of Th1/Th2 ratio, SOCS3, IL17 e IL35 levels in unexplained infertility? J Reprod Immunol 2014;103:53–8.
[43] Lee J, Choi BC, Cho C, Hill JA, Baek KH, Kim JW. Trophoblast apoptosis is increased in women with evidence of Th1 immunity. Fertil Steril 2005;83:1047–9.
[44] Donovan A, Hanrahan JP, Kummen E, Duffy P, Boland MP. Fertility in the ewe following cervical insemination with fresh or frozen–thawed semen at a natural or synchronized oestrus. Anim Reprod Sci 2004;84:359–68.