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Embryo transfer in sheep (*Ovis aries*), efficient and consistent framework in superovulation treatment

Abstract

Superovulation treatment is a key instance in Embryo Transfer, the Ovine Industry is an important target in Assisted Reproductive Technologies. In comparison with other ruminants, such as bovines, substantial differences in pathways of response to superovulation treatment in *Ovis aries* has been reported. We set out to evaluate superovulation protocols in sheep, published by different researchers and technicians, measuring outcome parameters to develop a highly repeatable methodological framework. Eight different protocols were installed in thirty-one post-pubertal embryo donors. Time and replace of intra vaginal pessaries, two sources of follicle-stimulating hormone, two estrous detection methods, and use, or not, GnRH, PGF2□ and LH in day of estrus, were rehearsed. Two variables were evaluated, ovarian response and embryo structures collected in-uteri, 5 to 6 days after heat detection.

Keywords: superovulation treatment, ovine industry, heat detection method, ovarian response, embryos structure collected, anovulatory follicles, corpus luteum early regression

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Abbreviations: SOVT, super ovulatory hormonal treatments; FOL, follicles; PAF, persistence of anovulatory follicles; CL, normal sized corpus luteum; ERCL, early regressed and small-sized CL; HD, heat detection; SC structures collected, AI, artificial insemination; ET, embryo transfer

Introduction

In sheep *(Ovis aries*), both Artificial Insemination (AI) and Embryo Transfer (ET), even In-Vitro Fertilization (IVF), have moderate industrial development, including countries with widespread production and technification, such as Australia, New Zealand or European Community.[1–3] Regarding ovarian response to super ovulatory hormonal treatments (SOVT), in comparison with other ruminants, such as bovines, substantial differences in pathways of response has been reported. Possible causes of these poor and variable response, is assigned to persistent anomalous ovarian structures, observed at the moment of collect embryos, it means, day four to six post estrous, at surface of both ovaries of the donor ewe.[2,4,7] These are: I° persistence of anovulatory follicles (PAF), observed as great size, diameter > 3mm, at flushing moment, day 4 to 6 after estrous; II ° early regression of the corpus luteum (CL) and presence of small-sized CLs (ERCL), diameter < 4mm, coexistent, or not, with PAF at same moment, at ovarian surface. Such anomalies, are probably producing serological imbalance estrogens progesterone, affecting uterine environment, triggering anovulation, early embryo mortality and reabsorption.[4–8] Even, this alterations, was correlated to the presence of larger follicles (>3mm), considered steroidogenic, observed by endoscopy just before starting the administration of gonadotropins and negatively correlated with the presence of small ones (< 2mm) at same treatment moment.[9–11]

As a PAF consequence, high serological levels of estrogens subsist during estrus, ovulation and early embryo development. This necessarily induces hostile uterine environment for both events, fertilization and embryo mature. Probably ERCL is an incremental factor of this frame, producing lowering P4 serological levels at these times.[7,9,11] For this reason, a third (III°) negative aspect occurs: the scarce, or null, association between ovarian response and uterine structures collected after uterine flush.[7,9,11] Whether they are associated phenomena or not, is reason for further studies, although, is very likely they have near relationship. In our experience, applying SOVT protocols, suggested by several authors, industry, and ours, in unpublished data, all profiles (I°, II° and III°) can be observed at ovarian surfaces during the period going from estrus until embryo collection day, namely: I°, one or several PAFs, cystic, after estrous and even 5-7 days later, refractories to ovulation hormonal treatments (i.e. GnRH-LH); II° ERCLs in day of flushing, in absence of expected ovarian response, measured as non-existent normal sized CLs (> 4mm) or, multiple small sizes CLs (more than three, < 3mm), coexisting with multiple PAFs (> 3-4mm); and III° erratic, or null, relationship between ovarian response and uterine oocyte-embryo structures collected.[7–9,11,14,15,16]

We set out to evaluate different SOVT protocols in sheep, measuring outcome parameters, to develop a highly repeatable methodological framework that can reverse these negative effects. Eight (8) different protocols SOVT were applied to thirty-one (31) post-pubertal embryo donors. In all of them, estrous was induced by intra-vaginal progesterone devices (IVD-Progespon Syntex), associated, or not, with luteolytic source (PGF2∝). The ovarian stimulation was performed using two different follicle-stimulating hormone (FSHp or eCG) fonts of; estrous detection was done with aproned or vasectomized rams, performing artificial insemination (AI) towards the end of estrus. Five to six days post AI, uterine flushing was carried out, counting and assessing the ovarian response, and relationship with oocyte-embryo collected structures. These eight protocols were chosen, among others, after our own experience and an extensive literature review reflecting unexplained poor TSOV response in the ovine model. At first, applying models suggested by literature and technicians of industry, (T I° to IV°), then (in T V° to VIII°), introducing physiological and pharmacological changes, seeking to improve the results measured as ovaries and oocyte structures observed at flushing day.[4–8,10,12]

The variables introduced into the SOVTs to this aim were: administration or not of GnRH or LH on the day of estrus; rams with an apron (AR) or vasectomized (VR), to heat detection/induction

(HD); time duration of the pessary insertion; reinsertion of the same; source of FSH (FSHp or eCG); application or not of PGF2∝ and its time; The assessment of different protocols were set out only with two dependent variables: 1) the ovarian response, (FOLs, CLs, PAFs, and ERCLs counted) and 2) the oocyte structures collected in-uteri (embryos, oocytes, pellucid zonas)

Material and methods

Thirty-one (31) post-pubertal, pure, or cross-breed Milchschaf, cyclic or a-cyclic, ewes, as embryo donors. Six rams of same breed were semen donor or estrous markers. At all management procedures, protocols of animal welfare were applied (CICUAL UBA, Argentina). Intravaginal polyurethane sponge devices (Progespon Syntex-Zoetis) impregnated with 60 mg Medroxyprogesterone Acetate (MAP) and prostaglandin F2∝ (sodium cloprostenol 263 µg /mL Ciclase-DL Syntex-Zoetis) were used for estrus synchronization. Equine chorionic gonadotrophin (eCG 200 IU/1mL, Novormon. Syntex-Zoetis), FSHp pituitary porcine extract (FSHp, LHp 100UI/mL, Pluset Calier Argentina.). EB, Estradiol benzoate, (benzoato de estradiol 1mg/mL, Syntex-Zoetis) as follicle stimulation treatments. To induce ovulation, synthetic GnRH agonist (Buserelina, acetate 0,00042 %, Gonasyn, Syntex-Zoetis) and human chorionic gonadotrophin, (hCG 500 UI/mL Ovusyn, Syntex-Zoetis) were administered. A laparoscopic or mid-ventral surgical approach was performed to expose female genital tract to record ovarian structures and accomplish uterine flushing for collect, evaluate and classify the oocyte-embryo structures 5-6 days after estrus. Eight different SOVT were assay:

Treatments

Treatment I° Decreasing FSHp dose & D14 DIVOUT+PGF2∝ D15-16 HD «Aprone»- D16 AI-D23 Flushing

Treatment I° consisted of: fourteen days' intravaginal device insert; decreasing FSHp dose, from eleventh to fourteenth day (D11, 2 x 35UI; D12 and D13, 4 x 32UI; D14 2 x 26UI) PGF2∝ dose at day fourteen, AM; heat detection (HD) with aproned ram (AR), fifteenth and sixteenth days and double laparoscopic intrauterine artificial insemination. Collect embryos on day twenty-three. Seven donors were treated (Figure 1).

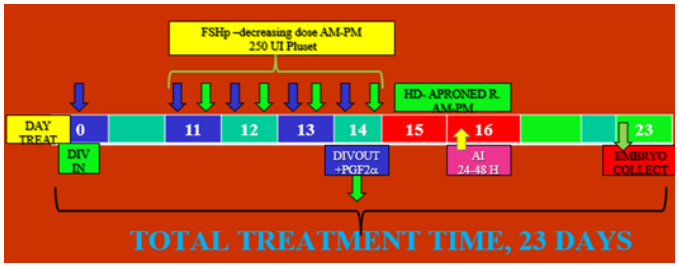

Figure 1 Treatment I°.

Treatment II° Decreasing FSHp dose & D14 DIVOUT+PGF2∝ HD «aprone» D16 AI+GnRH- D23 Flushing

Treatment II° consisted of: fourteen days' intravaginal device insert; decreasing FSHp dose from eleventh to fourteenth day (D11, 2 x 35UI; D12 and D13, 4 x 32UI; D14 2 x 26UI) PGF2∝ dose at day fourteen, AM; HD, with aproned ram, fifteenth and sixteenth days and double laparoscopic intrauterine artificial insemination, plus, GnRH on onset estrous. Collect embryos on day twenty-three. Four donors were treated. Was adjusted, in same model than TI°, though with GnRH administration at insemination day, to induce ovulation, in expectant of better perform of mature follicles (> 3mm) and reduce PAF occurrence at flushing day (Figure 2).

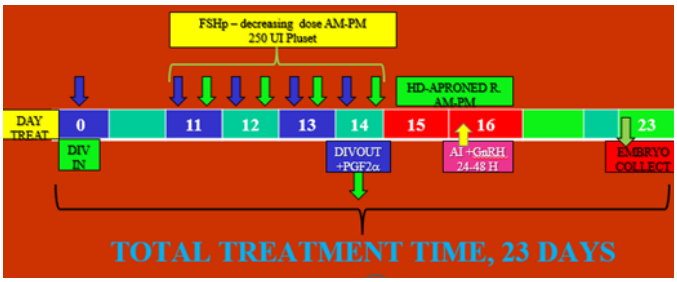

Figure 2 Treatment II°.

Treatment III° Decreasing FSHp dose & D14 DIVOUT+PGF2∝ HD «aprone» D16 AI+hCG-D23 Flushing

Treatment III° Four donors were treated. Progesterone intravaginal device was placed for fourteen days. Same source of FSH and model administration than treatments I° and II° were applied to stimulating ovaries. Decreasing FSHp serial doses from the eleventh to fourteenth day was performed (D11, 2 x 35UI; D12 and D13, 4 x 32UI; D14, 2 x 26UI). A PGF2∝ dose is given on day fourteen, AM. To induce ovulation, instead of GnRH, human chorionic gonadotrophin (hCG) was used, at insemination moment, expecting enhanced ovulation perform than TII°. Detection of estrus was carried out with a ram wearing an apron (AR), on the fifteenth and sixteenth days. Double laparoscopic intrauterine artificial insemination, plus, hCG was performed on day 16. Recorded of ovarian structures and embryo collection was performed by laparoscopic method on day twenty-three (Figure 3).

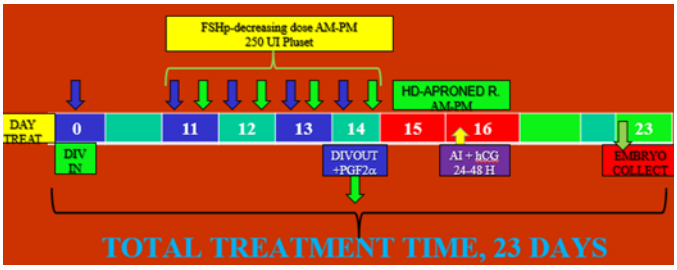

Figure 3 Treatment III°.

Treatment IV° Decreasing FSHp dose & D14-DIVOUT+PGF2∝-HD «vasectomized» D16 AI+GnRH-D23 Flushing

Treatment IV° Four donors were treated, with matrix similar to that T II°, except that vasectomized ram (VR) is used to estrous detect, consisted of: fourteen days' intravaginal device insert; decreasing FSHp dose from eleventh to fourteenth day (D11, 2 x 35UI; D12 and D13, 4 x 32UI; D14 2 x 26UI) PGF2∝ dose at fourteen day, AM; VR for HD, fifteenth and sixteenth days. Double laparoscopic intrauterine artificial insemination, plus, GnRH at first insemination time. Laparoscopic collect embryos and registration of ovarian structures on day twenty-three (Figure 4).

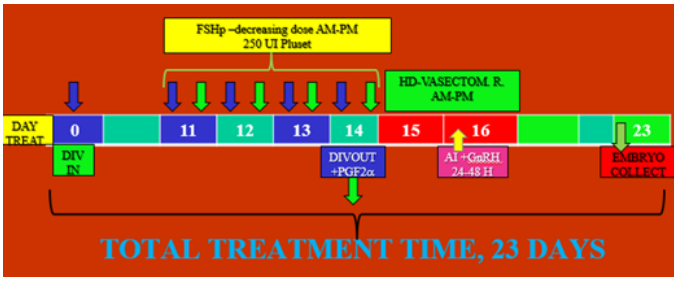

Figure 4 Treatment IV°.

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Treatment V° eCG single dose D14 + DIVOUT+PGF2a∝ HD «vasectomized» D15-16 double AI

Treatment V° Six donors were involved, consists in: fourteen days' intravaginal device insert; source of FSH was changed to a single injection of 1000 UI of equine chorionic gonadotrophin (eCG), at remove intra vaginal device day, 14th. A dose of estradiol benzoate (EB) and PGF2∝ were administered at same moment. Vasectomized ram (VR) makes estrous detection, days 15th and 16th, AM PM followed by laparoscopic insemination at double dose (D15 PM and D16 AM). Laparoscopic uterine flushing and ovaries structures examination, were performed six-seven days after estrous, day twenty-three of treatment (Figure 5).

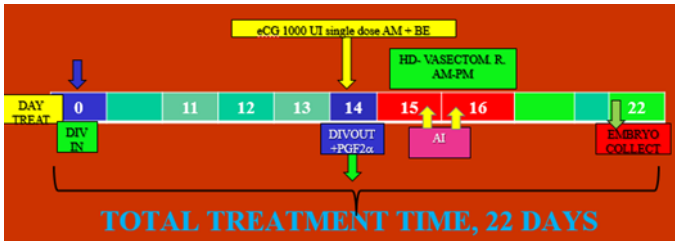

Figure 5 Treatment V°.

Treatment VI° eCG only dose D14 plus DIVOUT+PGF2∝ HD «vasectomized» D15-16 – D15 single AI

Treatment VI° Two donors were treated, consisted of: intravaginal device insert reduced to twelve days; estradiol benzoate (BE) and PGF2∝ given at moment of pessaries insertion. Administration of eCG, single dose (1000/1500 UI) was done eleventh day AM, 24 h before withdrawal intravaginal devices; HD was performed by vasectomized ram (VR) on day14th, AM-PM. IA was accomplished on day 14th PM by single semen dose by intrauterine administration, in addition to a GnRH hormonal treatment. Six days later, laparoscopic collect embryos by flushing the uterus was done and response of the ovaries was registered (Figure 6).

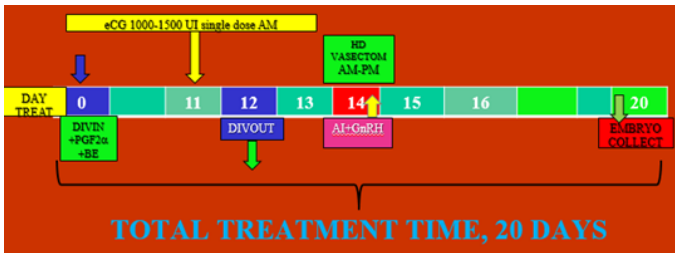

Figure 6 Treatment VI°.

Treatment VII°: D7 RE-DIVIN D12 single dose eCG plus PGF2∝ D14 DIVOUT; D14,15,16 HD «Vasectomized» D15 single AI; D21 Embryo collect

Treatment VII° Two donors were treated, intra vaginal devices insertion changes were introduced, consists in: day '0', intravaginal device insert, day 7th replacement of intravaginal device with a new one; ovaries stimulation was done with 1250UI eCG in a single dose, on day 12th, in addition with PGF2∝ administration at same moment; day 14th, two days later, second and definitive removal of intravaginal device; detection of estrus (HD), days 14, 15, 16th by vasectomized ram (VR); single laparoscopic intrauterine artificial insemination on day 14th; ovarian response and uterine flushing for embryos collection were verified by surgical approach on day 21 of treatment (Figure 7).

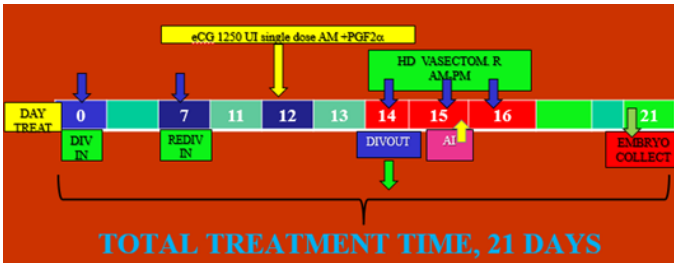

Figure 7 Treatment VII°.

Treatment VIII°: D7 RE-DIVIN; D12 single dose eCG; D14 IVDOUT; D14,15,16 HD «Vasectomized» D15 single AI plus GnRH; D21 Embryo collect

Treatment VIII° Two donors were treated. The aim of this treatment was evaluate the possible ovulation effect of GnRH at insemination time, in a similar matrix to that of VII protocol, given the large number (7) of PAFs found in one of its donors. Protocol consisted of: day 0, insertion of the intravaginal device; day 7th, replacement of the intravaginal device; day 12th AM eCG single dose (without PGF2∝); heat detection days 14th, 15th and 16th per vasectomized ram (VR) Day 15th single intrauterine artificial insemination plus administration of a GnRH dose. Ovarian response, and uterine flushing for embryo collection were verified by surgical approach on day 21 (Figure 8).

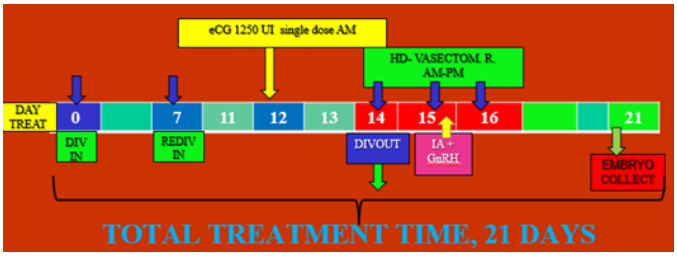

Figure 8 Treatment VIII°.

Statistical Analysis

Data for presence of PAFs and ERCLs at ovaries groups, were analyzed using unpaired Student's *t*-test and two tailed Mann Whitney test procedures, for independent samples, using *GraphPad* ^{TM} statistical software.

Results

Treatment efficiency were evaluated at flushing moment, days 4-6 post IA, by ovarian response and oocyte-embryo structures collected at uterine lumen. Results are summarized at Table 1.

Treatment I°: Of seven donors treated in TI°, one gives 10 SC, one deliver 2 SC and two donors give 1 SC; (average 2 SCs per donor) and 12 normal sized CLs (average 0,857 per ovary/donor). Another three ewes, do not produce embryos or other structures at the time of collection, day 5-6 after estrous. Nevertheless, all donors show ovaries structures as PAF (3,428 per donor) or ERCL (2,14 per donor).

Treatment II° Four donors were performed, no one yield SC. Instead, 20 PAF were observed at ovaries (5 per donor) and 8 ERCLs (mean 2 per donor). No normal sized CLs were founding also.

In treatment III° From two of the three donors treated, two embryo structures (SCs) was obtained at collection day. Five ERCLs (mean 1,66 per donor) and three normal sized CLs (1/donor) were observed at ovaries surfaces. Instead, nineteen PAF in two from three ewes (19/4- mean 9,50/donor) were found.

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Table 1 Embryo and Ovarian structures at collection day in different treatments and estrous detection

TTM	DTTD	STCCT	PAF	ERCL	CLs	TTMENT adjustment
I°	7	14	24	15	12	FSHp, APRONED +PGF
II°	4	0	20	8	0	FSHP APRONED+PGF+GnRH
III°	4	2	19	5	3	FSHp+APRONED+PGF+ hCG
IV°	4	8	7	3	10	FSHp VASECTOM.+PGF+ GnRH
V°	6	3	17	10	6	eCG VASECTOM.+PGF+BE
VI°	2	0	3	1	0	eCG VASECTOM.+BE+PGF+GnRH
VII°	2	6	7	2	8	eCG D12 -REDIVIN-VASECTOM.+PGF
VIII°	2	0	12	1	3	eCG D12 REDIVIN VASECTOM.+GnRH
Total	**31**	**33**	**109**	**45**	**42**	

Table 1: Column 1, TTN: treatment number; Column 2, DTTD: ewe donors treated per treatment; Column 3, STCCT: oocyte or embryo structures collected, (SC), independent of which one (no fertilized oocyte, empty zona or embryo) at uterine lumen; Column 4: PAF refers persistent anovulatory follicles, (> 3mm), at flushing day; Column 5, ERCL: small sized CLs, (defined as pale, avascular, diameter less than 3mm) or ERCL; Column 6, CLs: summarize normal sized, (>3mm), functional CLs. Column 7, TTMENT ADJUSTMENT: summarizes treatment adjustments. Treatment details in text; PAF persistent anovulatory follicles (>3mm); ERCL, early regressed corpus luteum (<3mm); CLs, normal sized corpus luteum (>3mm); Aproned or Vasectomized refers estrous detection method.

Treatment IV° Four ewes were involved. In two donors 8 embryo structures (SC) were collected at the uterine lumen. In these donors, 8 normal sized CLs (> 4 mm), 1 ERCL and no PAF were recorded on the ovarian surfaces of these donors. In another two donors, no oocyte or embryos structures were found at flushing, instead, the cortex of the ovaries, showed 7 PAF (3,5/donor), 2 ERCL (1/donor) and 2 normal sized CLs (1/donor).

In Treatment V° were implicated six ewes, two of them gave three normal embryos. These ewes, at the ovaries, showed 4 normal sized CLs (2/donor), 5 ERCLs (2,5/donor), 6 PAFs (3/donor). No oocyte or embryo structures were collected in the other four sheep, these ones, at ovarian cortex exhibit 11 PAFs, (2,75/donor) 5 ERCLs (1,25/donor), 2 normal sized (>4mm) CLs (0,5/donor) .

Treatment VI° from two donors involved no oocyte or embryo structures were collected at uterine lumen, notwithstanding the above, one ERCL (0,5/donor) and three PAFs (1,5/donor) were recorded in ovarian surfaces of both ewes.

In treatment VII° from two ewes implicated, one donor exhibit 6 normal sized CLs (>5mm) 1 ERCL (< 4mm) and no follicles in her ovaries. In this female, the uterine collection yields 5 oocyte-embryo structures. The other sheep shows 1 ERCL, 7 PAF (>3 mm, 4 <3mm) in both ovaries, at flushing one embryo was collected. In both donors PAF, 3,5/donor, ERCL 1/donor, normal sized CLs 3/donor.

Treatment VIII° Two ewes were treated in this matrix, adding to VII° model, an ovulation hormonal treatment (GnRH) to reduce PAFs (7) observed in this last model. The ovarian response was contradictory to our forecasts, no oocyte-embryo structures were founded, however 12 PAFs (6/donor) and 1 ERCL (0,5/donor), was found, along with 3 normal sized CLs (1,5/donor)

For all the models used, only 12 of 31 treated sheep (38.7%) responded by giving oocyte-embryo structures in lumen of the uterus at the embryo collection time, well below what happens in cattle. Furthermore, only 5 of these 12 donors, (41,66%), did not exhibit steroidogenic, anovulatory, cystic, follicles (PAF) at same time. Moreover, of all 31 sheep treated, only 5, (16,12 %) did not show PAF at ovaries surface this phase (Table 1). If in the ewe, effective super ovulation treatment must be considering, only, when more than three ovulatory structures are found at uterine lumen at collection time, in our results, only 3 from 31 donors treated, (9,67%), could be mentioned with active response. Induction-detection of estrus using VR, compared to AR, in similar TSOV treatments (II° y IV°) significantly reduce PAF per ewe/ovary (P v 0,0407,). ERCLs ratio shows some difference between both procedures, VR 0,46/0,21 versus AR 0,53/0,26 per ewe/ovary, but statistically no significant (P v 0,1367,) The fact that VR accomplish natural coitus, with penis vaginal introduction, in our experience, furthermore, shortened estrous manifestation pattern extent.

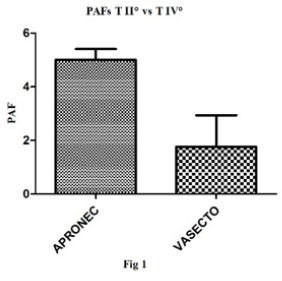

Fig 1

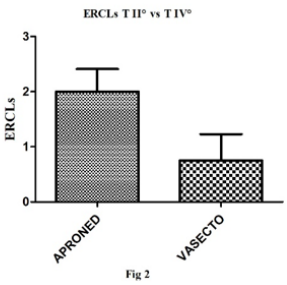

Fig 2

If the amount of PAFs, on the surface of the ovary, is measured on the day of embryo collection, taking separately the group of donor ewes whose heat was detected with an aproned ram (n15) versus the group whose heat was detected with a vasectomized ram (n16), the difference between groups is statistically significant (63/15 - 46/16- P v 0.0135-). When measuring ERCLs recorded on the ovaries, the same day treatment, in both groups (aproned versus vasectomized), the difference (28/15 - 17/16- P v 0,1064-), although higher in aproned group versus vasectomized group, it is not statistically significant.

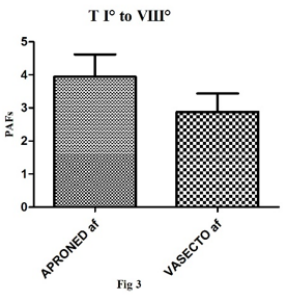

Fig 3

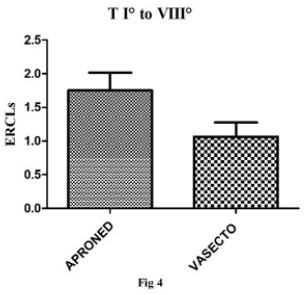

Fig 4

Inconsistent results were obtained spending GnRH or hCG to induce ovulation at insemination time (estrus day), to provoke dominant follicles maturation-ovulation, and thus, reduce PAFs incidence or increased normal sized CLs, present on ovarian surfaces at collect moment. Controversial outcomes, also, were found by mean of use PGF2∝ dose; whether administered at the time of insertion or withdraw pessaries, no difference was observed. In protocol that

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suggests replace DIV on the seventh day, if PGF2∝ is given 48H prior to remove second device, exhibit some impact. The same could be said for BE, certain positive influence would be observed if it were given at DIV pessaries withdrawal, as shown in T V°, associated with eCG and PGF2∝ administration. Regarding the insertion length of intra vaginal progesterone device, shortening them from 14D to 12D, did not give outstanding result (T VI°) However, replacing it halfway through of protocol duration, seventh day, apparently works, probably by raise progesterone serum level during implant period (TVII° and VIII°) In TVII° more oocyte structures were collected, fewer ERCLs and more normal sized CLs were recorded, and fewer incidence of PAFs was found. The source of FSH, equine or porcine, made no difference, however, some authors report evidence that serial injections, as is usual in porcine or ovine extracts, work worst compared to a single dose.[7]

Discussion

In our experience, performance of ovulation, in SOVT in *Ovis aries* is crucially low, compared with *Bos taurus* or *Oryctolagus cuniculus* [(15)]. Different groups, from research or industry, all over the countries, find similar results, currently. Clearly dissimilar hormonal profiles still exist in the ewe, in contrast to other farm animals, this fact conspires against ovulation, fertilization and early-embryo uterine environment at the super ovulated ewe donor.[4,5,7,11,13,14] In scientific literature, different authors have diverse approaches to explain this asymmetry. These are: corporal condition, stress, follicular waves status, steroidogenesis disturb during synchronization and super ovulation treatment.[4,5,7–9,10,13] Probably all of them could be involved, but, necessarily some of them must be more important than others.

Our results clearly highlight the appearance of large, cystic, anovulatory follicles (>3mm, PAF), signaling as highly steroidogenic, at days 5, 6, or 7 post-estrous. This ovarian structures do not respond to ovulatory hormonal sources such as GnRH or LH, during or after estrus. For the consequent reason of high estrogenic serological levels at this critical moment, the uterine environment must be hostile to fecundation process, or early embryo development. Another structural consequence of this fact, would be the consistent appearance of ERCL, it means, small sized CLs (< 3mm), which produce lower levels of progesterone, being another of the restrictive factors for an embryo-friendly uterine environment. In superovulated ewe, after estrous, comparative studies of estrogen and progesterone level, showed disturbs and disequilibrium to the expected serum profile. It was pointed out that this hormonal disorders produce gradual regression of CLs, with the consequent decrease in progesterone circulation and estrogen/progesterone imbalance, which aggravates the physiological situation.[9,11]

Presence of small antral follicles (<3mm) in the ovaries at the onset of TSOV, was be positive correlated with response to a SOVT treatment; large steroidogenic follicles (>6mm) present in this same moment, is negative correlated to the final number of transferable embryos at collection date.[10,13] The reason why, mature follicles avoid ovulation on time, during or immediately post estrus, has been cause of speculation.[4–7,9,10,13] Time and level of LH peak, studied in different super ovulated ewe protocol, shows remarkable variations, induced, among others, by modus and moment of FSH source administration.[7] These alterations, in addition to studies that have not yet done so, in relation to the time and moment of synthesis of FSH and LH receptors by granulosa cells or oocyte cytoplasmic membrane, should be necessarily studied to clarify why, not only is there no response of this follicles to an endogenous LH surge, if not also to an exogenous administration of GnRH, or, an external source of LH as well. Probably the synthesis profile assumed by these involucrate cells it is similar to that of the cystic follicles cells present in dairy and beef cattle. Some recent studies in sheep advise roles of bone morphogenetic proteins (BMPs) and FecB gene, a regulator factor of these protein, playing some responsibility as a regulator of ovulation rates, but its effect in TSOV ewes, remains unclear.[17,18]

Single FSH injection, (eCG alone, or combined with FSHp) anticipates, and produce major LH surge.[7,13] In our work, anticipating FSH dose, administered in single injection, 48 hours before the pessaries withdrawal, sort better the estrous synchronization, and, produce less ERCLs and PAF at embryos collect time. Several dose administrations of GnRH agonist, two weeks prior to install progesterone sponge, or serial GnRH antagonist treatment during the same, suggested by some authors[10,13] was discarded, because the fact of controversial and variables results among authors and breeds, and, in many cases, cause of endocrine disturb that causes poor quality embryos, in addition to increased cost, animal handling and stress.[9,10,13]

Conclusion

More studies are necessaries, specifically to restraint presence of PAFs and ERCLs at ovarian surface at embryo collection date. Therefore, the challenge for consistent results must be to study protocols that restrict endogenous small follicles growing during the progesterone implant for estrus synchronization, enabling the recruitment and stimulate the increase of mature dominant ovulatory follicles at estrus day probably by: 1) increasing serum progesterone levels during days 7 to 12 post DIV insertion, replacing the intravaginal device seven days after starting treatment, achieved by re-insertion pessaries on day 6-7 of treatment; 2) restrict FSH treatment to a one-day/dose, regardless of the hormonal source (eCG, FSHo, FSHp or their combinations); 3) detect estrus allowing coitus with intromission, performing it during three days after sponge withdrawal, using vasectomized ram instead of aproned one; 4) single FSH dose administration, must be preferred 48 h prior to remove pessaries, associated, at same time, with a luteolytic agent; 5) before performing the procedures, the donor ewe's previous reproductive performance, body condition, and handling stress, should be considered. This, would include, particularly, feed and environment to holding donors.

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Conflicts of interest

The authors declare no conflict of interest exists.

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