Maturitas 78 (2014) 17–21

Contents lists available at ScienceDirect

Maturitas

journal homepage: www.elsevier.com/locate/maturitas

Review

Management of infertility in women over 40

Rosalie Cabry[a,1], Philippe Merviel[a,1], Andre Hazout[b,2], Stephanie Belloc[b,2], Alain Dalleac[b,2], Henri Copin[a,1], Moncef Benkhalifa[a,*]

[a] Reproductive Medicine and Medical Cytogenetics Department, Regional University Hospital and School of Medicine, Picardie University Jules Verne, CGO, 124 rue Camille Desmoulins, 80054 Amiens, France
[b] Eylau/Unilabs Laboratory, Reproductive Biology Unit, 55 Rue Saint Didier, 75016 Paris, France

ARTICLE INFO

Article history:
Received 2 December 2013
Received in revised form 19 February 2014
Accepted 21 February 2014

Keywords:
Female over 40
Fertility potential
Assisted reproductive technology

ABSTRACT

Women's fertility potential is declining with age because of multiples intrinsic and extrinsic factors such as life style, oxidative stress and/or endocrine disruptors and is affecting the ability of these women to conceive naturally. This declining fertility potential and the late age of motherhood is increasing significantly the number of patients consulting infertility specialists. Different strategies of investigation and management are proposed to patients over 40 in order to overcome their infertility and improve the live birth rate in these patients.

Intra Uterine Insemination (IUI) in women over 40 is associated with a low rate of ongoing pregnancy and IUI should not therefore be offered always as the first line of treatment. When the predictive factors are positive IVF/ICSI seem to be good alternatives until 43 years of age. Customized ovarian stimulation and flexible laboratory methods such as in vitro maturation (IVM), preimplantation genetic diagnosis (PGD), embryo vitrification and transfer after thawing in subsequent natural or artificial cycles can improve the success rate of ART in patients over 40. Meanwhile, oocyte and embryos donation remain good options for patient over 40 with a bad prognosis and can lead to successful ongoing pregnancies until 45 years of age. Ovarian tissue cryopreservation, oocyte vitrification at the germinal vesicle (GV) stage or metaphase II stage present a breakthrough for fertility preservation but the ideal age for starting fertility preservation is still debated as well as the minimum number of oocytes to be vitrified in order to optimize the chances of pregnancy when needed at an older age.

This manuscript reports the results of our own experience from patients older than 40 in the light of the published data and discusses the different therapeutic alternatives which can be proposed to patients over 40 consulting ART centres.

Contents

* Corresponding author. Tel.: +33 322533677; fax: +33 322533679.
E-mail addresses: Cabry.Rosalie@chu-Amiens.fr (R. Cabry), Merviel.Philippe@chu-Amiens.fr (P. Merviel), hazout.andre@hotmail.com (A. Hazout), Stephanie.belloc75@gmail.com (S. Belloc), Alain.Dalleac@Unilabs.com (A. Dalleac), Copin.Henri@chu-Amiens.fr (H. Copin), Benkhalifamoncef78@gmail.com (M. Benkhalifa).
[1] Tel.: +33 322533677; fax: +33 322533679.
[2] Tel.: +33 143140879811; fax: +33 142277306.

http://dx.doi.org/10.1016/j.maturitas.2014.02.014
0378-5122/

1. Introduction

The number of women over 40 years of age seeking infertility treatment has been steadily increasing in the past decades due to postponing childbearing in career women as well as the desire of pregnancy in couples starting a second family [1]. In addition to various treatments to enhance ovulation in these women, the percentage of women over 40 requiring assisted reproductive technology (ART) has increased significantly from 10% to 15% in the 2000s [2] to 20% to 25% in 2009 [3]. This emphasizes the importance of optimizing the medical and clinical management strategies in these patients treated with various forms of ART.

Studies have shown that the effectiveness of ART techniques decrease with female age and this is in accordance with the natural fertility performance after 40 years of age. Women over 40 must be informed about the low success rate as well as the high risks related to maternal age in pregnancy. These includes an increased risk of hypertension, preeclampsia, gestational diabetes, placenta praevia, placental abruption, caesarean section, genomic disorders, prematurity, low foetal birth weight and neonatal morbidity [4].

Consequently, various alternatives are being offered to young women in order to preserve their fertility potential, increase their take home baby rate while minimizing the risks and adverse effects of pregnancy when this is achieved after 40 years of age. These include different treatments to preserve fertility such as food supplements and hormonal therapy as well as assisted reproductive technologies combined or not to pre implantation genetic diagnosis where the repeated collection and vitrification of the oocytes earlier in life for use in later years is becoming a real option for those women before resorting to oocyte or embryo donation.

The objective of this manuscript is to present our own experience in managing and preserving the fertility of women older than 40 in the light of the published literature and emphasize the different therapeutic alternatives that can be proposed to these women to improve their fertility potential.

2. ART for women over 40: facts and clinical data

The negative impact of maternal age on IVF/ICSI success rate is mainly due to the diminished quantity and quality (maturation and competence) of collected oocytes regardless of the stimulation protocol [5]. Published studies report a clinical pregnancy rate of 10–15% in women over 40 undergoing IVF or ICSI [6]. In our centre, from January 2007 to December 2011, a total of 500 IVF/ICSI cycles in women over 40 were attempted leading to 425 oocytes retrievals, an ovarian stimulation failure of 15%. The average number of collected oocytes per retrieval attempt was 8.6 while no oocytes were collected in 1.5% of the cycles. The fertilization rate was 50.2% with an average of 3.4 embryos per cycle obtained on day 3. Of these embryos, 41.9% were of grade A according the classification of Terrioux et al. [6]. From our study, from 425 patients who had oocyte retrieval, 334 proceeded to embryo transfer (78%) with an average of 2.1 embryos per transfer. The biochemical pregnancy was 17.6%, the ongoing pregnancy was 8.9% while the live birth rate was 7.4% (see the detail of our study in Table 1). Our transfer cancellation rate was therefore 22% and the miscarriage rate was 49%.

These data from our centre are in line with other published studies. In the study of Tsafir et al., 381 patients older than 40, were started on ART therapy. Of these, 83.4% proceeded to ovum pick up and 62.6% had embryo transfers. The clinical pregnancy rate was 7.3%, the rate of spontaneous abortions was 33% and the live birth rate was 4.7% [7]. Similarly, Bongain et al. studied 194 IVF cycles in a group of patients with a mean age of 40.9 years. They reported a mean number of 4.6 oocytes per retrieval and a 3.6% live birth rate [8].

Several studies have also reported low birth rates from patients over 40. This observation is caused mainly by early miscarriages (33–42%) and embryo aneuploidies [4,5,9]. In a series of patients with a mean age of 45.4 years who underwent IVF/ICSI treatment, Spandorfer et al. [10] observed that 85.3% of patients miscarried after implantation. These data confirm that the fertility potential is declining with age and particularly after 43 years. From standard IVF cycles, Ron-El et al. [11] obtained a clinical pregnancy rate of 14%, 9%, 26% and 0% in women aged 41, 42, 43 and 44, respectively while the delivery rate was 7%, 2%, 13% and 0% in the same respective groups. In another study of 843 cycles in women aged 42 and over, Ciray et al. [12] obtained a clinical pregnancy rate of 7.7% in those aged 42, 5.4% in those aged 43, 1.9% at age 44, while no pregnancy occurred in 54 cycles in patients over 45. In an analysis of live births after 3 cumulative IVF/ICSI cycles with embryo transfers, Klipstein et al. [9] obtained a clinical pregnancy rate of 25, 3% in women aged 40 versus 18.8% at age 41–42, 9.6 at age 43 and 1.6 at age 44. All these studies show clearly that there is a cut-off for ART efficiency after 44.

Since age is a major limiting factor for fertility potential, it is important to evaluate the predictive factors of pregnancy in women over 40. The ovarian reserve assessment tests using the antral follicles count, the plasma concentration of anti-Müllarian hormone (AMH), FSH and oestradiol have been proposed as methods to predict the risk of ovarian failure. However, most of the authors correlate ovarian failure to the number of retrieved oocytes, with patients providing more than 5 oocytes considered as having a good prognosis with preserved ovarian activity. On the other hand, patients producing less than 5 oocytes are labelled as poor responders with a diminished ovarian reserve [8]. From 1114 embryo transfers in patients over 40 with a mean age of 41.6 years, Ciray et al. [12] obtained 11% of ongoing pregnancies with 5.9% of live birth from patients who produced a minimum of 5 matures oocytes versus 24.3% of pregnancy and 15% of live birth from patients who produced a minimum of 6 mature oocytes. Our own series showed a significant difference in clinical and ongoing pregnancy rates per transfer between patients who provide more than 5 oocytes compared to those who produce 5 or less ($P < 0.001$) (Table 1).

The age of the woman seems to be a better predictive factor than the level of plasma FSH. From a cohort of patient aged from 40 to 43 years, with an FSH lower than 15 IU and a minimum of 6 antral folicles, Van Disseldorp et al. [13] obtained a clinical pregnancy rate of 8% and a cumulative pregnancy rate of 17% after 3 cycles of treatment. When patients over 40 years of age with plasma FSH levels of > 15 IU were compared to patients older than 41 with FSH levels < 15 IU, Van Rooij et al. [14] reported better implantation and ongoing pregnancy rates in the younger group. This observation

Table 1
Relation between the number of collected oocytes and clinical outcome from patients over 40's after a minimum of 2-repeated IVF/ICI failure.

Number of collected complex cumulus cells	≤5 Oocytes	6–14	≥15
Average maternal age (year)	41.5 ± 1.1a	41 ± 1c	40.7 ± 0.8b
Average paternal age (year)	42.3 ± 5.7	41.7 ± 5.6	41.2 ± 5.9
Infertility duration (year)	3.4 ± 1.8d	4 ± 3.2f	5.5 ± 4e
Day 3 FSH level (UI/l)	8 ± 2.7 g	6.8 ± 2.1i	6.5 ± 1.6 h
Number of cycles	**181**	**260**	**59**
Rank of attempt	2.2 ± 1.3j	2.5 ± 1.5k	2.6 ± 1.5
Total administrated FSH (UI)	5055 ± 1545o	3667 ± 1514q	2953 ± 1142p
Oestradiol the day of hCG (pg/ml)	1446 ± 363r	2101 ± 960t	2685 ± 375s
Cancellation rate (%)	23.2	11.1	6.7
Completed cycles via OPU	**139**	**231**	**55**
Average collected cumulus	3.4 ± 1.3u	9.3 ± 2.5w	18.8 ± 3.6v
Average métaphase II	2.5 ± 1.4u	7 ± 2.9w	13.7 ± 4.4v
Fertilization rate (%)	46.4	51.6	48.9
Cleavage rate (%) 50.1	50.1	54.4	50.5
Mean transferred embryos/transfer	1.7 ± 1a′	2.2 ± 1c′	2.5 ± 1b′
Clinical pregnancy/transfer (%)	**7.9d′**	**21.7e′**	**18.7**
Implantation rate/embryo (%)	5	10.4	7.4
Ongoing pregnancy/transfer (%)	**3.4f′**	**11.1g′**	**10.4**
Live birth/transfer (%)	**2.2h′**	**10.1i′**	**6.2**

a-c: < 0.0001; a-b: < 10^6; b-c: < 0.02; d-f: < 0.03; d-e: < 0.001; f-e: < 0.01; g-i: < 10^4; g-h: < 10^4; j-k: < 0.05; o-q: < 10^9; q-p: < 0.001; o-p: < 10^9; r-t: < 10^9; t-s: < 10^9; r-s: < 10^9; u-w et w-v et u-v: < 10^9;a′-c′: < 0.0001; a′-b′: < 10^4; d′-e′: < 0.01; f′-g′: < 0.05; h′-i′: < 0.05; j′-k′: < 10^9.

demonstrates that the maternal age remain the main limiting factor regardless of the plasma levels of FSH.

If the number of collected oocytes after pick up is a significant factor limiting the chance of success for patient older than 40, the remaining question is: "what would be the most suitable ovarian stimulation protocol to optimize the quantity and the quality of oocyte cumulus complexes retrieved?" The study of Van Rooij et al. [14] comparing the use of long and short GnRH agonist protocols reported that the long stimulation protocol produces more oocytes (5.3 versus 3.3) but there were no significant difference in the number of transferred embryos and in the delivery rate. On the contrary, in women older than 40, Sbracia et al. [15] obtained a higher number of oocytes and embryos with a better clinical pregnancy rate per transfer in 281 cycles treated with the short protocol compared to 283 cycles using the long protocol. GnRH antagonists protocols have also been tried in these patients. However, results show that patients older than 40 with a diminished ovarian reserve and submitted to GnRH antagonist or short GnRh agonist (± micro doses) protocols obtain the same results in term of ongoing pregnancy [16]. In our experience it seems that in patients with an adequate ovarian reserve, GnRH long agonist protocol leads to a better clinical pregnancy rate. There are no clear data showing the superiority of one gonadotropin or one protocol over another. However, a recent meta-analysis [17] showed that the supplementation of various ovarian stimulation protocols with recombinant LH seems to improve the implantation and clinical pregnancy rates in patients over 35 years of age. Mild stimulation associated to low dose of gonadotropins under antagonist is also an interesting alternative for patients with poor ovarian reserve [18]. Compared to classical protocols this alternative may produce more good quality embryos, better implantation and pregnancy rates when the same number of embryos is transferred [19]. Based on this observation, mild stimulation could be a beneficial option to patients over 40.

The number of transferred embryos is also a predictor of pregnancy. Tsafir et al. [7] reported a significantly higher clinical pregnancy rate in patients who received 3 embryos per transfer and Klipstein [9] found a significantly higher birth rate when 2 embryos were transferred versus the transfer of a single embryo. The same conclusion was observed when 3 embryos were transferred versus only 2 and 4 versus 3, etc. It was suggested that 5 embryos is the best number of embryos to be transferred to reach an acceptable pregnancy and live birth rate in women over 40 [20]. Revisiting the literature, only Ciray et al. [12] did not find a statistically difference after the transfer of 3 or more embryos.

It also seems logical to observe the relation between the indication of ART and the success rate and whether this is performed for tubal, male or idiopathic indications. In our experience we did not find any significant difference in terms of pregnancy rates according to the indication.

3. Intra uterine insemination for women over 40

Infertile women over 40 years of age with bilateral tubal patency, acceptable ovarian reserve and without major sperm disorders can be offered intrauterine insemination. In a series of 82 IUI cycles in patients aged between 40 and 42 years old, Haebe et al. [21] reported a live birth rate of 9.8% but the analysis of 24 cycles from patients older than 43 years showed a delivery rate of only 4.2%. This study confirms the rational of offering IUI to patients over 40 but not to those over 43 years of age. However, offering IUI to patient over 40 in cases of idiopathic indication is still a debatable point and Frederick et al. [22] reported a low clinical pregnancy rate of 5% and a live birth rate of 1.4% in those patents. Conflicting studies were published by several authors. For example, Corsan et al. [23] studied 4 groups of patients and obtained an ongoing pregnancy rate of 9.6%, 5.2%, 2.4% and 0% in patients aged 40, 41, 42 and over 43 years, respectively. In this cohort Corsan et al. [23] reported a spontaneous miscarriage rate of 34.4%. From a homogeneous group of patients over 40 undergoing IUI, Andersen et al. [24] obtained an ongoing pregnancy rate of 9.7%, while Lamarche et al. [25] reported 10.5% and 5.8% of live births reported a 10.5% and 5.8% of live birth. In 2007, Custers et al. [26] proposed a statistical model to establish a score to assess the chances of pregnancy after IUI. In this model, the main limiting factor was the woman's age, but other parameters used were the cause and the duration of infertility, the uterine characteristics and the couple's history.

4. Oocytes and embryo donation

In infertile women over 40 years of age and treated with IVF/ICSI, using donated oocytes from young patients can be offered as a good efficient alternative to improve their pregnancy and delivery rates [27]. The Spanish experience [28] shows a clinical pregnancy rate of reported 53.4% per cycle with a delivery rate of 42.6%. The cumulative pregnancy rate after 4 cycles of embryos transfer is

nearly 94%, showing no effect of the recipient patient's age on the efficiency of oocyte donation programme. These data should be analyzed carefully because there is always a risk of a negative effect of the endometrial receptivity and the immunological status of aged patients on the implantation rate and ongoing pregnancy loss.

These high success rates can be explained by the efficiency of patient preparation protocols prior to embryo transfer, the experience of the specialist managing the donation and the borderline age of recipients. In fact, in a large meta-analysis, Vernaeve et al. [29] found lower chances of pregnancy in recipients over 45 years of age. The advantage of oocyte or embryo donation to patient over 40's is the absence of the risks associated with ovarian stimulation. However, obstetrical complications such as gestational diabetes, pre-eclampsia and thrombosis should be considered in older patients even with oocyte or embryo donation [30]. Today the remaining question is: "What is the cut off age to minimize the risks of complications in those patients?" and this has not so far been answered with a clear consensus. From the follow up of 22 patients over 50 who became pregnant after oocyte donation, Sauer et al. [27] reported obstetrical complications in 47% of them. In another study, the follow up of 123 pregnancies achieved through oocyte donation in elderly women reported that 63% of patients over 45 needed hospitalization compared to 22% in those aged between 45 and 49 [31]. In practice embryo donation is more commonly used in cases of combined male and female infertility, especially if specific genomes and/or epigenome decays are diagnosed and not corrected after multiple treatments. Both oocyte and embryos donation possibilities are different from country to country and raise ethical and legal issues such as financial compensation of the donors, their anonymity and the waiting time of enrolled patients in the donation programme.

5. Self fertility preservation

There is a growing shortage of oocytes and embryos available for donation because of the fertility declining trends in the general population. In addition, couples treated with IVF/ICSI are increasingly being reluctant to donate oocytes and embryos to other couples because of moral and/or ethical reasons. Consequently, self fertility preservation has been developed to preserve oocytes or ovarian tissue of women for use in later years of their lives. With the efficiency of cryobiology technology and mainly of vitrification, early self oocyte banking appears to be a solution for later motherhood and for the decline in fertility related to hormonal disorders or biological ageing. The ideal age of oocyte collection and cryopreservation is still debated as the predictors of ovarian failure are poorly understood. Oocyte collection can be proposed at a young age but no later than 30–35 years during which the ovarian reserve is still satisfactory and when the opportunity of natural pregnancy is still present.

The debatable points are: (a) how should we manage patients to minimize the complication risks of ovarian stimulation, (b) how many stimulation cycles can be proposed to patients in relation to age and ovarian reserve, and (c) what is the minimum number of oocytes that can be vitrified and used in subsequent IVF/ICSI cycles to improve the success. According Stoop et al. [32] nearly 22 vitrified metaphase II oocytes are needed to achieve pregnancy in women aged between 23 and 37 years. Knowing that the average number of collected oocytes is 8 per stimulation cycle in this age group, this implies that 2–3 ovarian stimulation and oocyte pick up cycles are needed to achieve a live birth. For patients aged 38–43 years, a minimum of 55 vitrified metaphase II oocytes is needed to achieve a pregnancy [32]. For all ages Cobo et al. [33] recommend that at least 12 metaphase II vitrified oocytes are needed to achieve clinical pregnancy in an oocyte cryopreservation programme.

Ovarian tissue cryopreservation is another alternative. Today, it is mainly offered to cancer patients. Its application for social reasons, later motherhood or to rescue declining fertility is still rare but could be an alternative to classical ovarian stimulation by the cryo preservation of Germinal Vesicles oocytes followed by in vitro maturation and/or Metaphase II oocyte vitrification. The effects of long term vitrification and the maternal age limit for the use of vitrified material is still debated as well as the relation between ovarian ageing and the biological age of the patient. Meanwhile, precautions should be taken concerning endometrium receptivity and immuno-tolerance to achieve pregnancy with a lower risk of complications.

6. Conclusions

Many women are currently postponing motherhood to an older age for various reasons. It is possible that some changes concerning the life style, food habits, special care to reduce the effect of oxidative stress and pollution as well as hormonal therapies may minimize the risk of physiological disorders and keep a better fertility potential over 40 years of age. Data from our own IVF/ICSI programme and from other groups are consistent with the meta-analysis of Amstrong et al. [34] showing that the success rate from IUI is below 5% while IVF and ICSI remain ideal options by giving a live birth rate of 10–15%. Patients should be clearly informed that IUI cannot be proposed as a first line option even with good sperm parameters and should also be informed about the risk of spontaneous miscarriage and complications. Ovarian tissue and oocyte preservation are becoming an option for patients with early risk of fertility decline. In case of ovarian reserve failure with good prognostic of implantation, oocyte and embryos donation offer rescue alternatives prior to adoption.

Infertile women over 40 need help to achieve pregnancies and live births. This includes financial, legal and ethical support to minimize complication risks during the clinical management and to improve the clinical outcome. Finally, the community should have the wisdom not to abuse ART programmes after a number of IVF/ICSI failures and to orient patient to other options such as oocyte/embryo donation or adoption.

Recent studies have also reported the negative effects of toxic agents and endocrine disruptors on follicular development and fertility potential leading to their decline with age between generations [35,36]. Oxydative stress and reactive oxygen species can also affect the oocyte environment and early embryo development potential [37].

Contributors

Authors contribute equally to manuscript preparation

Competing interests

None of the authors have a conflict of interest or competing interests.

Provenance and peer review

Commissioned and externally peer reviewed.

Funding

This manuscript is funded by Jules Verne University and Unilabs France.

Acknowledgements

We would like to thank Professor Hassan Sallam. MD. Ph. D from Oby & Gyn Dept. Alexandria University. Egypt, for manuscript reading, comments and improvement.

References

[1] Craig BM, Donovan KA, Fraenkel L, Watson V, Hawley S5, Quinn GP. A generation of childless women: lessons from the United States. Womens Health Issues 2014;24(1):21–7.
[2] Nyboe Andersen A, Gianaroli L, Nygren KG, European IVF-monitoring programme, European Society of Human Reproduction and Embryology. Assisted reproductive technology in Europe, 2000. Results generated from European registers by ESHRE. Hum Reprod 2004;19(March (3)):490–503.
[3] Ferraretti AP, Goossens V, Kupka M, et al. Assisted reproductive technology in Europe, 2009: results generated from European registers by ESHRE. Hum Reprod 2013;28(September (9)):2318–31.
[4] Suchartwatnachai C, Wongkularb A, Srisombut C, Choktanasiri W, Chinsomboon S, Rojanasakul A. Cost-effectiveness of IVF in women 38 years and older. Int J Gynecol Obstet 2000;69:143–8.
[5] Toner J. Age = egg quality, FSH level = egg quantity. Fertil Steril 2003;79:491.
[6] Terriou P, Giorgetti C, Auquier P, et al. Value of an embryo score to predict implantation. Contracept Fertil Sex 1996;24:657–60.
[7] Tsafir A, Simon A, Revel A, Reubinoff B, Lewin A, Laufer N. Retrospective analysis of 1217 IVF cycles in women aged 40 years old and older. RBM Online 2007;14:348–55.
[8] Bongain A, Castillon JM, Isnard V, Benoit B, Donzeau M, Gillet JY. In vitro fertilization in women over 40 years of age. A study on retrospective data for eight years. Eur J Obstet Gynecol Reprod Biol 1998;76:225–31.
[9] Klipstein S, Regan M, Ryley D, Goldman M, Alper M, Reindollar R. One last chance for pregnancy: a review of 2705 in vitro fertilization cycles initiated in women age 40 years and above. Fertil Steril 2005;84:435–45.
[10] Spandorfer S, Bendikson K, Dragisic K, Schattman G, Davis O, Rosenwaks Z. Outcome of in vitro fertilization in women 45 years and older who use autologous oocytes. Fertil Steril 2007;87:74–6.
[11] Ron-El R, Raziel A, Strassburger D, Schachter M, Kasterstein E, Friedler S. Outcome of assisted reproductive technologie in women over the age of 41. Fertil Steril 2000;74:471–5.
[12] Ciray HN, Ulug U, Tosun S, Erden HF, Bahceci M. Outcome of 1114 ICSI and embryo transfer cycles of women 40 years of age and over. Reprod Biomed Online 2006;13:516–22.
[13] Van Disseldorp J, Eijkermans MJC, Klinkert ER, Te Velde ER, Fauser BC, Broekmans FJM. Cumulative live birth rates following IVF in 41–43 year old women presenting with favourable ovarian reserve characteristics. RBM Online 2007;14:455–63.
[14] Van Rooij I, Bancsi L, Broekmans F, Looman C, Habbema J, Te Velde E. Women older than 40 years of age and those with elevated follicle-stimulating hormone levels differ in poor response rate and embryo quality in in vitro fertilization. Fertil Steril 2003;79:482–8.
[15] Sbracia M, Farina A, Poverini R, MorGia F, Schimberni M, Aragona C. Short versus long gonadotropin-releasing hormone analogue suppression protocols for superovulation in patients ≥40 years old undergoing intracytoplasmic sperm injection. Fertil Steril 2005;84:644–8.
[16] Pu D, Wu J, Liu J. Comparisons of GnRH antagonist versus GnRH agonist protocol in poor ovarian responders undergoing IVF. Human Reprod 2011;26:2742–9.
[17] Hill M, Levens E, Levy G, et al. The use of recombinant luteinizing hormone in patients undergoing assisted reproductive techniques with advanced reproductive age: a systematic review and met-analysis. Fertil Steril 2012;97:1108–14.
[18] Zarek S, Muasher S. Mild/minimal stimulation for in vitro fertilization: an old idea that needs to be revisited. Fertil Steril 2011;95:2449–55.
[19] Ubaldi F, Rienzi L, Baroni E, et al. facts about mild ovarian stimulation. RBM Online 2007;14:675–81.
[20] Combelles CMH, Orasanu B, Ginsburg ES, Racowsky C. Optimum number of embryos to transfer in women more than 40 years of age undergoing treatment with assisted reproductive technologies. Fertil Steril 2005;84:1637–42.
[21] Haebe J, Martin J, Tekepety F, Tummon I, Shepherd K. Success of intrauterine insemination in women aged 40–42 years. Fertil Steril 2002;78:29–33.
[22] Frederick JL, Denker MS, Rojas A, et al. Is there a role for ovarian stimulation and intra uterine insemination after age 40. Hum Reprod 1994;9:2284–6.
[23] Corsan G, Trias A, Trout S, Kemmann E. Ovulation induction combined with intra uterine insemination in women 40 years of age and older: is it worthwhile. Hum Reprod 1996;11:1109–12.
[24] Andersen AN, Gianorelli L, Felberbaum R, et al. Assisted reproductive technolohy in Europe, 2001.Results generated from european registersby ESHRE. Hum Reprod 2005;20:1158–76.
[25] Lamarche C, Levy R, Felloni B, et al. Assisted reproductive techniques in women aged 38 years or more. Gynecol Obstet Fertil 2007;35:420–9.
[26] Custers IM, Steures P, Van der Steeg JW, et al. External validation of a prediction model for an ongoing pregnancy after intrauterine insemination. Fertil steril 2007;88:425–31.
[27] Sauer MV, Paulson RJ, Lobo RA. Pregnancy in women 50 or more years of age: outcomes of 22 consecutively established pregnancies from oocyte donation. Fertil Steril 1995;64:111–5.
[28] Remohi J, Yalil S, Gartner B, Simon C, Gallardo E, Pellicer A. Pregnancy and birth rates after oocyte donation. Fertil Steril 1997;67:717–23.
[29] Vernaeve V, Reis Soares S, Budak E, Bellver J, Remohi J, Pellicer A. Clinical factors associated with the outcome of oocyte donation. Gynecol Obstet Fertil 2007;35:1015–23.
[30] Michalas S, Loutradis D, Drakakis P, et al. Oocyte donation to women over 40 years of age: pregnancy complications. Eur J Obstet Gynecol Reprod Biol 1996;64:175–8.
[31] Simchen MJ, Yinon Y, Moran O, Schiff E, Sivan E. Pregnancy outcome after age 50. Obstet Gynecol 2006;108:1084–8.
[32] Stoop D, De Munck N, Jansen E, et al. Clinical validation of a closed vitrification system in an oocyte donation program. RBM Online 2012;24:180–5.
[33] Cobo A, Diaz C. Clinical application of oocyte vitrification: a systematic review and meta-analysis of randomized controlled trials. Fertil Steril 2011;96:277–85.
[34] Amstrong S, Akande V. What's the best treatment option for infertile women aged 40 and over. J Assit Reprod Genet 2013;30(5):667–71.
[35] Bhattacharya P, Keating A. Impact of environmental exposures on ovarian function and role of xenobiotic metabolism during ovotoxicity. Toxicol Appl Pharmacol 2012;261:227–35.
[36] Uzumcu M, Zachow R. Developmental exposure to environmental endocrine disruptors: consequences within the ovary and female reproductive function. Reprod Toxicol 2007;23:337–52.
[37] Babuška V, Cedíková M, Rajdl D, et al. Comparison of selective oxidative stress parameters in the follicular fluid of infertile women and healthy fertile oocyte donors. Ceska Gynekol 2012;77:543–848.